Conserved Molecular Underpinnings and Characterization of a Role for Caveolin-1 in the Tumor Microenvironment of Mature T-Cell Lymphomas

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Conserved Molecular Underpinnings and Characterization of a Role for Caveolin-1 in the Tumor Microenvironment of Mature T-Cell Lymphomas

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Abstract

Neoplasms of extra-thymic T-cell origin represent a rare and difficult population characterized by poor clinical outcome, aggressive presentation, and poorly defined molecular characteristics. Much work has been done to gain greater insights into distinguishing features among malignant subtypes, but there also exists a need to identify unifying characteristics to assist in rapid diagnosis and subsequent potential treatment. Herein, we investigated gene expression data of five different mature T-cell lymphoma subtypes (n = 187) and found 21 genes to be up- and down-regulated across all malignancies in comparison to healthy CD4+ and CD8+ T-cell controls (n = 52). From these results, we sought to characterize a role for caveolin-1 (CAV1), a gene with previous description in the progression of both solid and hematological tumors. Caveolin-1 was upregulated, albeit with a heterogeneous nature, across all mature T-cell lymphoma subtypes, a finding confirmed using immunohistochemical staining on an independent sampling of mature T-cell lymphoma biopsies (n = 65 cases). Further, stratifying malignant samples in accordance with high and low CAV1 expression revealed that higher expression of CAV1 in mature T-cell lymphomas is analogous with an enhanced inflammatory and invasive gene expression profile. Taken together, these results demonstrate a role for CAV1 in the tumor microenvironment of mature T-cell malignancies and point toward potential prognostic implications.

Introduction

Mature T-cell lymphomas are a heterogeneous group of malignancies representing 10–15% of all non-Hodgkin’s lymphomas with 17,850 cases diagnosed in the United States between 2003–2012 [1, 2]. Mature T-cell lymphomas are characterized by aggressive growth, generally poor clinical outcome, and only a paucity of reported genetic abnormalities [3–6]. Currently, the World Health Organization recognizes a number of mature T-cell lymphoma subtypes, including: angioimmunoblastic T-cell lymphoma (AITL), anaplastic large cell lymphoma (ALCL),
adult T-cell leukemia/lymphoma (ATLL), hepatosplenic T-cell lymphoma (HSTL), and peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) [7].

The state of research on mature T-cell lymphomas aims to enhance the recognition of molecular subtypes, thereby improving diagnostics; ultimately these produce improved prognostic models to aide in treatment [8–13]. Advances in the area of diagnostics lead to increased classification rates diverging from PTCL-NOS [8, 10, 11], which has been considered a “wastebasket” category [7]. While molecular diagnostics to improve the classification rates of T-cell lymphoma subtypes have obvious value in terms of targeted treatment, understanding unifying characteristics of a group of malignancies sharing an extra-thymic cell-of-origin is warranted. Therefore, an enhanced understanding of the shared molecular underpinnings of neoplasms of T-cell origin could lead toward the development of novel combinatorial therapies and information regarding the basic biology of mature T-cell lymphomas.

The goal of this study was to conduct an expansive meta-analysis (sometimes termed mega-analysis) [14] of microarray data on mature nodal and splenic T-cell lymphomas to construct a gene signature shared across all subtypes. To this end, we mined the NCBI GEO DataSets (Table 1) for chip-matched, mature T-cell lymphoma samples (n = 187) and healthy CD4⁺ and CD8⁺ T-cell controls (n = 52) with focus on genes annotated to function in T-cell receptor signaling, T-cell co-stimulation, T-cell homeostasis, and T-cell differentiation in the gene ontology (GO) directory to mitigate background from the stromal compartment. The abovementioned genetic findings were then corroborated at the protein level using human biopsies of mature T-cell lymphoma cases (n = 130 core biopsies from n = 65 unique cases).

Herein, presented are the findings of the genetic analysis with an increased focus on caveolin-1 (CAV1), a gene seen heterogeneously upregulated across all T-cell lymphoma subtypes, mimicking data observed in chronic lymphocytic leukemia, a B-cell neoplasm [15, 16, 17]. These data support a correlation between CAV1 and the promotion of an inflammatory and invasive phenotype in mature T-cell lymphomas. Future research will be required to determine whether CAV1 is directly involved in the process.

### Table 1. Publically available, chip-matched GEO DataSets of mature T-cell lymphomas and healthy CD4⁺ and CD8⁺ T cells utilized for gene expression profiling.

| GEO Accession | Samples Utilized (n-value) | PMID |
|---------------|---------------------------|------|
| GSE43017      | Healthy Samples: CD4⁺ T cell (5) | N/A |
| GSE6338       | Malignant Samples: PTCL-NOS (28), AITL (6), ALCL (6) Healthy Samples: CD4⁺ T cell (5), CD8⁺ T cell (5) | 17304354 |
| GSE61399      | Healthy Samples: CD4⁺ T cell (3) | N/A |
| GSE14879      | Malignant Samples: ALCL (9) | 19657361, 24376854 |
| GSE19067      | Malignant Samples: HSTL (4) | 21052088 |
| GSE19069      | Malignant Samples: AITL (37), ALCL (30), ATLL (13), PTCL-NOS (50) Healthy Samples: CD4⁺ T cells (1) | 19965671 |
| GSE25087      | Healthy Samples: CD4⁺ T cell (3) | 21164017 |
| GSE31773      | Healthy Samples: CD4⁺ T cell (8), CD8⁺ T cell (8) | 21917308 |
| GSE36769      | Healthy Samples: CD4⁺ T cell (4) | 23778140 |
| GSE49954      | Healthy Samples CD4⁺ T cell (5), CD8⁺ T cell (5) | 24130824 |
| GSE57944      | Malignant Samples: HSTL (4) | 25057852 |

AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; ATLL, adult T-cell leukemia/lymphoma; HSTL, hepatosplenic T-cell lymphoma; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified; PMID, PubMed identifier

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Results

Construction of a shared T-cell compartment gene signature across mature T-cell lymphomas

In order to delineate a shared T-cell compartment signature among a diverse grouping of mature T-cell lymphomas, we conducted differential expression analyses of the five different mature T-cell lymphoma subtypes collected, focusing on GO annotations specific to T-cell biology. We analyzed each T-cell lymphoma subtype separately, with a final manual compilation of genes found to be differentially expressed across all subtypes. This analysis revealed an up-regulation of 6 genes (namely \textit{CAV1}, \textit{CCNB2}, \textit{ENAH}, \textit{PSEN2}, \textit{THY1}, \textit{TNFRSF21}) (Fig 1A) and a down-regulation of 15 genes (namely \textit{BCL10}, \textit{CD3G}, \textit{CD5}, \textit{FOXP1}, \textit{IL23A}, \textit{ITK}, \textit{LAT}, \textit{PAG1}, \textit{PDE4B}, \textit{PRKCQ}, \textit{RICTOR}, \textit{STAT5A}, \textit{TNFRSF14}, \textit{TSC22D3}, \textit{UBASH3A}) (Fig 1B) that were observed to be differentially expressed (p < 0.001, FDR < 0.001) in all T-cell lymphoma subtypes (Table 2). Further, 52 genes were found to either be up- or down-regulated in at least 3 out of 5 of the analyzed subtypes (S1 Table).

To show the robust nature of these findings, the 21 pan-regulated genes were used for class prediction using the top-scoring pairs (TSP) method [18]. This pairwise comparison method selects a family of gene pairs and classifies samples based on a decision involving the comparison of the ratio of mRNA abundance for selected gene pairs [18]. Of the 21 candidate genes, TSP scored the expression ratio of \textit{CAV1} (Fig 1C) and \textit{BCL10} (Fig 1D) to hold the greatest magnitude of change. Using the \textit{CAV1-BCL10} classifier, T-cell lymphoma samples were classified with 98.4% sensitivity and 88.5% specificity (S2 Table). These results suggest that the inverse expression of \textit{CAV1} and \textit{BCL10} may assist in the diagnosis of mature T-cell lymphomas, and can be attributable to all analyzed subtypes.

Stratification of mature T-cell lymphomas by expression of Caveolin-1

As previously reported, higher \textit{CAV1} expression in B-cell lymphomas leads to a more aggressive disease [15, 16] with an inferior overall survival among patients with higher \textit{CAV1} expression [15]. Therefore, we decided to further investigate the differences between \textit{CAV1}-High and \textit{CAV1}-Low expressing mature T-cell lymphoma samples in an effort to characterize the impact of \textit{CAV1} on the tumor microenvironment (TME). Analysis of \textit{CAV1} expression using 212097_at probe signal intensity revealed that all mature T-cell lymphoma subtypes analyzed have a higher mean expression of \textit{CAV1} (p < 0.001, FDR < 0.001) as compared to healthy \textit{CD4+} and \textit{CD8+} T-cell controls (Fig 1C). However, as expected, there exists a heterogeneous expression of \textit{CAV1} amongst the malignant samples. To stratify samples into the abovementioned groups, healthy controls and malignant samples were pooled, respectively. Moreover, a \textit{CAV1}-Low expression threshold was calculated based on the distribution of \textit{CAV1} expression in healthy samples (Fig 2A). Using this threshold, 67% (125/187) of mature T-cell lymphoma samples were classified as \textit{CAV1}-High (Fig 2B). For each T-cell lymphoma subtype: 77% (33/43) of AITL samples, 47% (21/45) of ALCL samples, 85% (11/13) of ATLL samples, 75% (6/8) of HSTL samples, and 69% (54/78) of PTCL-NOS samples were classified as \textit{CAV1}-High.

The \textit{CAV1}-High tumor microenvironment is enriched in genes involved in the immune response, chemokine/cytokine activity, and invasive genes

As shown above in Fig 2, mature T-cell lymphoma samples highly express \textit{CAV1}, with 67% of samples harboring expression that is 2 standard deviations above mean \textit{CAV1} expression in \textit{CD4+} and \textit{CD8+} T cells. However, 33% of samples express \textit{CAV1} within a healthy range, with
A no one-subtype exclusively harboring high CAV1 expression. To investigate the impact of higher CAV1 expression on the TME (i.e. the whole tissue sample), we used the class comparison among groups of arrays method. To expand our analysis to the TME, yet reduce inter-malignancy variation, only genes significantly correlated to CAV1 expression were considered (see Materials and Methods). Of the 7,170 genes analyzed, we report that 2,624 genes were differentially expressed (p < 0.001, FDR < 1% at 99% confidence) between the two classes, 1,758
(67%) of which were overexpressed in the CAV1-High group. For analysis, we input the fold-changes values from the genes found differentially expressed into the PANTHER gene analysis tool [19, 20]. PANTHER enrichment analyses reveal the CAV1-High TME to be enriched in genes annotated to be involved in chemokine/cytokine activity, invasion (migration/motility/movement), and immune processes (Table 3). Chemokine/cytokine activity featured enrichments in biological processes, molecular functions, and the chemokine protein family. Invasive activity was found to harbor enrichments in biological processes and enrichment of the integrin signaling pathway (S1 Fig). Immune processes featured enrichments in biological processes. Furthermore, overrepresentation analysis revealed significant (p < 0.05) overrepresentation of genes upregulated in CAV1-High conditions to be involved in the PANTHER pathways for inflammation mediated by chemokine and cytokine signaling (S2 Fig) and angiogenesis (S3 Fig). As CAV1 is overexpressed in mature T-cell lymphomas, the tumor microenvironment adopts a gene expression profile indicative of increased inflammation and metastatic potential, as demonstrated above. This phenotype suggests a correlation between CAV1 expression and increased inflammation in the tumor microenvironment of T-cell lymphomas. Increased

Table 2. Genes significantly up- or down-regulated across all mature T-cell lymphoma subtypes as compared with healthy CD4+ and CD8+ T-cell controls.

| Gene Name                        | Symbol   | Chromosomal Location | AITL Fold-Change | ALCL Fold-Change | ATLL Fold-Change | HSTL Fold-Change | PTCL-NOS Fold-Change |
|----------------------------------|----------|----------------------|------------------|------------------|------------------|------------------|----------------------|
| Caveolin-1                       | CAV1     | 7q31.1               | 16.33            | 12.52            | 38.17            | 34.17            | 16.33                |
| Cyclin B2                        | CCNB2    | 15q22.2              | 7.55             | 8.93             | 13.99            | 9.65             | 7.55                 |
| Thy-1 cell surface antigen       | THY1     | 11q23.3              | 13.65            | 12.98            | 9.46             | 9.65             | 13.65                |
| Tumor necrosis factor receptor   | TNFRSF21 | 6p21.1               | 6.38             | 6.27             | 5.34             | 9.30             | 6.38                 |
| superfamily member 21            |          |                      |                  |                  |                  |                  |                      |
| Enabled homolog                  | ENAH     | 1q42.12              | 5.62             | 5.36             | 2.52             | 22.63            | 5.62                 |
| Presenilin 2                     | PSEN2    | 1q42.13              | 2.94             | 4.77             | 2.26             | 3.39             | 2.94                 |
| 3g molecule, gamma (CD3-TCR      | CD3G     | 11q23                | -3.01            | -6.81            | -1.65            | -2.02            | -3.00                |
| complex)                         |          |                      |                  |                  |                  |                  |                      |
| Tumor necrosis factor receptor   | TNFRSF14 | 1p36.32              | -1.49            | -1.58            | -1.69            | -1.99            | -1.49                |
| superfamily member 14            |          |                      |                  |                  |                  |                  |                      |
| RPTOR independent companion of   | RICTOR   | 5p13.1               | -2.42            | -4.36            | -1.75            | -2.25            | -2.42                |
| MTOR, complex 2                  |          |                      |                  |                  |                  |                  |                      |
| Linker for activation of T-cells | LAT      | 16p11.2              | -2.91            | -3.61            | -1.92            | -3.76            | -2.91                |
| CD5 molecule                     | CD5      | 11q13                | -4.12            | -6.55            | -2.10            | -17.27           | -4.11                |
| Interleukin 23, alpha subunit p19| IL23A    | 12q13.3              | -3.26            | -3.50            | -2.23            | -4.51            | -3.26                |
| IL2-inducible T-cell kinase      | ITK      | 5q31-q32             | -3.67            | -10.78           | -2.36            | -2.70            | -3.67                |
| Signal transducer and activator  | STAT5A   | 17q11.2              | -2.14            | -2.11            | -2.50            | -2.63            | -2.14                |
| of transcription 5A              |          |                      |                  |                  |                  |                  |                      |
| TSC22 domain family, member 3    | TSC22D3  | Xq22.3               | -5.66            | -6.63            | -2.56            | -4.07            | -5.66                |
| Ubiquitin-associated and SH3      | UBASH3A  | 21q22.3              | -2.43            | -4.27            | -2.86            | -6.97            | -2.43                |
| domain-containing protein A       |          |                      |                  |                  |                  |                  |                      |
| Protein kinase C theta            | PRKCI    | 10p15                | -2.98            | -8.26            | -3.58            | -2.92            | -2.98                |
| Forkhead box P1                   | FOXP1    | 3p14.1               | -4.90            | -3.96            | -3.69            | -3.40            | -4.90                |
| Phosphoprotein membrane anchor   | PAG1     | 8q21.13              | -3.81            | -2.32            | -4.30            | -3.69            | -3.81                |
| with glycosphingolipid microdomains 1|       |                      |                  |                  |                  |                  |                      |
| B-cell CLL/lymphoma 10            | BCL10    | 1p22                 | -4.96            | -7.82            | -6.54            | -7.15            | -4.96                |
| Phosphodiesterase 4B, cAMP-specific | PDE4B   | 1p31                 | -4.21            | -3.55            | -7.44            | -3.42            | -4.21                |

AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; ATLL, adult T-cell leukemia/lymphoma; HSTL, hepatosplenic T-cell lymphoma; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified.

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Fig 2. T-cell lymphomas can be divided into CAV1-High and CAV1-Low subgroups. (A) Boxplot of normalized 212097_at (CAV1) probe signal between pooled healthy controls and malignant samples. Continuous line represents median value, discontinuous line represents mean. Red line represents threshold for CAV1-High or CAV1-Low expression determination and is set 2 standard deviations above the healthy T-cell mean value. (B) Classification of CAV1-High and CAV1-Low samples according to expression threshold. Solid bars and above-bar values represent percentage of CAV1-High samples per subtype. 67% (125/187) of all malignant samples were classified as CAV1-High. 77% (33/43) of AITL samples, 47% (21/45) of ALCL samples, 85% (11/13) of ATLL samples, 75% (6/8) of HSTL samples, 69% (54/78) of PTCL-NOS samples.

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Table 3. PANTHER enrichment analysis of genes upregulated in the CAV1-High tumor microenvironment.

| Immune Enrichment | GO Annotation                                      | Number of Genes | Over/Under | p-value |
|--------------------|-----------------------------------------------------|-----------------|------------|---------|
| Biological Process | Regulation Of Leukocyte Activation                  | 59              | +          | 2.10E-02|
|                    | Activation Of Immune Response                        | 65              | +          | 3.32E-02|
|                    | Immune Effector Process                              | 76              | +          | 1.03E-04|
|                    | Positive Regulation Of Immune Response               | 81              | +          | 2.61E-02|
|                    | Regulation Of Immune Response                        | 125             | +          | 4.92E-02|
|                    | Regulation Of Immune System Process                  | 189             | +          | 2.85E-04|
|                    | Immune Response                                      | 194             | +          | 1.00E-04|
| Chemokine/Cytokine Enrichment | Cell Chemotaxis                                       | 24              | +          | 9.08E-05|
| Molecular Function | Regulated Chemotaxis                                 | 30              | +          | 2.81E-02|
|                    | Cytokine-Mediated Signaling Pathway                  | 65              | +          | 1.33E-03|
|                    | Inflammatory Response                                | 68              | +          | 1.21E-02|
|                    | Cellular Response To Cytokine Stimulus               | 86              | +          | 7.25E-05|
|                    | Response To Cytokine                                 | 106             | +          | 3.97E-06|
| Pathway:           | Integrin Signaling Pathway                           | 36              | +          | 4.44E-02|

\[^{1}\] p-values calculated using the Bonferroni correction

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inflammation could lead to increased tumor burden and recruitment of non-neoplastic cells [21, 22], potentially leading to a more aggressive disease.

Immunohistochemical analysis on an independent sampling of mature T-cell lymphomas

To investigate our genetic findings at the protein level, we purchased LY6161 high-density lymphoma and normal lymph node tissue arrays from U.S. Biomax, Inc. These tissue arrays contained $n = 25$ normal lymph nodes and $n = 130$ core biopsies from $n = 65$ unique, mature T-cell lymphoma patients (8 AITL, 14 ALCL, and 43 PTCL-NOS patients, respectively). Separate tissue array slides were stained with antibodies directed towards: BCL10, CAV1, CD90 (Thy-1), GILZ (TSC22D3), in addition to standard hematoxylin and eosin staining. Summarized analysis of immunohistochemical staining can be found in Table 4, detailed analysis of individual samples can be found in S3 Table, with representative imaging found in Fig 3.

For BCL10 staining, 88% (7/8) of AITL samples, 93% (13/14) of ALCL samples, and 91% (39/43) PTCL-NOS samples were scored positive for lymphoid staining. 2 ALCL samples and 1 PTCL-NOS sample were scored light in comparison to other positive samples in terms of lymphoid staining. 1 PTCL-NOS sample was scored with light stromal/non-lymphoid staining, and 1 AITL sample, 1 ALCL sample, and 3 PTCL-NOS samples were scored negative for lymphoid staining. For CAV1 staining, 63% (5/8) of AITL samples, 43% (6/14) of ALCL samples, and 58% (25/43) of PTCL-NOS samples were scored positive for lymphoid staining. 1 AITL sample was negative for CAV1 staining. 2 AITL samples, 8 ALCL samples, and 18 PTCL-NOS samples were positive for stromal/non-lymphoid staining. For GILZ staining, 100% (8/8) of AITL samples, 93% (13/14) of ALCL samples, and 93% (40/43) PTCL-NOS samples were scored positive for lymphoid staining. 2 AITL samples, 1 ALCL sample, and 7 PTCL-NOS samples were scored light in comparison to other positive samples in terms of lymphoid staining. 1 ALCL sample was scored with stromal/non-lymphoid staining, and 3 PTCL-NOS samples were scored negative for presence of stain. For CD90 staining, 75% (6/8) of AITL samples, 36% (5/14) of ALCL samples, and 42% (18/43) of PTCL-NOS samples were scored positive for lymphoid staining. 6 ALCL samples and 13 PTCL-NOS samples were scored positive for stromal/non-lymphoid staining, and 2 AITL samples, 3 ALCL samples, and 12 PTCL-NOS samples were scored negative for presence of stain.

The results from protein staining of the TMAs support the genetic findings two-fold. First, as the GEP data are publically derived, routine qPCR validations are unavailable and a secondary confirmation of protein analysis is needed. The protein findings support the GEP results through reaffirming that the GEP data are originating from tumor lymphocytes within the samples and are not strictly artifacts of stromal components, as the biopsy samples used for the GEP analyses were neither flow sorted nor laser micro-dissected and we can determine the localization of staining based on histology of the TMA. These findings are supported by the staining patterns displayed in Fig 3 showing positive staining in tumor lymphocytes as opposed to solely stromal and/or non-lymphocyte staining. Secondly, as the TMA samples are completely distinct in patient origin to those used in the GEP we can see that the GEP results, this shows the strength of these findings in that they are consistent between genomic and protein expression across a large sample size of cases of lymphoma.

Long non-coding RNA 273 is computationally predicted to interact with members of a CAV1-interaction network

Strikingly, an uncharacterized long, intergenic non-coding RNA (LINC00273) was found to be expressed 3-fold higher in CAV1-Low T-cell lymphoma samples compared to CAV1-High (Fig
As long non-coding RNAs have been implicated in RNA-protein binding complexes, we sought to investigate if LINC00273 represented a potential RNA-protein interaction (RPI) partner with CAV1 and CAV1-interacting proteins, as CAV1 expression was the basis upon which the abovementioned groups were stratified. Using STRING v10, we determined the predicted CAV1-interactions networks for genes upregulated in both CAV1-High (Fig 4B) and CAV1-Low groups (Fig 4C). Of the 1,758 genes upregulated in CAV1-High samples, 38 proteins were modeled to interact (either direct, or indirect) with CAV1 (Fig 4B). The CAV1-High interaction network featured 100 protein-protein interactions, of which the protein constituents were found to be enriched in GO biological processes for regulation of cell differentiation \((p = 2.7 \times 10^{-4})\), cell migration \((p = 4.2 \times 10^{-4})\), cell proliferation \((p = 7.2 \times 10^{-4})\), apoptotic processes \((p = 2.87 \times 10^{-4})\), and the KEGG TGF-beta signaling pathway \((p = 1.9 \times 10^{-6})\). Conversely, only 4 proteins of the 866 genes upregulated in CAV1-Low samples were modeled to interact with CAV1 (Fig 4C). This protein network featured 4 protein-protein interactions and was

| Diagnosis                          | Sample # | Median Age | % Male | BCL10^2 | CAV1^2 | GILZ^2 | CD90^2 |
|------------------------------------|----------|------------|--------|---------|--------|--------|--------|
| Anaplastic large cell lymphoma     | 14       | 45         | 50% (7/14) | 93% (13/14) | 43% (6/14) | 93% (13/14) | 36% (5/14) |
| Angioimmunoblastic T-cell lymphoma | 8        | 50         | 63% (5/8)  | 88% (7/8)  | 63% (5/8)  | 100% (8/8) | 75% (6/8)  |
| Peripheral T-cell lymphoma-NOS     | 43       | 41         | 53% (23/43) | 91% (39/43) | 58% (25/43) | 93% (40/43) | 42% (18/43) |

^1 Age reported in years
^2 Percentages reflect staining observed in lymphoid cells

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found to be enriched for the GO biological processes of fibroblast growth factor receptor signaling \((p = 1.91 \times 10^{-4})\) and ERBB signaling \((p = 5.46 \times 10^{-4})\).

Using available prediction methods for RPI, we sought to achieve a consensus computational prediction concerning the interaction potential of \(LINC00273\) in CAV1-interaction networks. The IncPro [23], RPISeq (both Random Forrest [RPISeq-RF] and Support Vector Machine [RPISeq-SVM] output) [24], and RPI-Pred [25] programs were used in the above-mentioned RPI-prediction analysis. Of the 43 proteins evaluated for RPI, IncPro scored 100% of potential proteins as interacting, RPISeq-RF scored 100% as interacting, RPISeq-SVM scored 7% (3/43) as interacting, and RPI-Pred scored 100% as interacting. Detailed results can be found in Table 5. These computational data support a potential interaction between \(LINC00273\) and members of a CAV1-interaction network. However, the exact functional consequences of these interactions are not fully understood. Potentially, \(LINC00273\) may serve as a scaffold, increasing the stability or proximity of the CAV1-interacting proteins, or may hold regulatory properties over its interacting proteins.

Fig 4. Long non-Coding RNA 273 is computationally predicted to interact with members of a CAV1-interaction network. (A) Boxplot of normalized AFFX-HUMRGE/M10098_5_at \((LINC00273)\) probe signal for CAV1-Low and CAV1-High expression groups. Continuous line represents median value, discontinuous line represents mean value. (B) CAV1-interacting proteins upregulated in the CAV1-High expression group. Interaction network viewed in String evidence view, connection lines are defined by in-Fig box. (C) CAV1-interacting proteins upregulated in the CAV1-Low expression group. Interaction network viewed in String evidence view, connection lines are defined by in-Fig box.

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Table 5. Computational predictions for RNA-protein interactions between LINC00273 and CAV1-interacting proteins.

| Protein                                         | Symbol | UniProt Sequence ID | IncPro¹ | RPISeq-RF² | RPISeq-SVM³ | RPI-Pred         |
|------------------------------------------------|--------|---------------------|---------|------------|-------------|-----------------|
| Adenylate cyclase 2*                            | ADCY2  | Q08462-1            | 76.6134 | 0.8        | 0.346       | Interaction Possible |
| Adrenergic, beta, receptor kinase 1*            | ADRBK1 | P25098-1            | 91.8466 | 0.75       | 0.343       | Interaction Possible |
| Amyloid beta (A4) precursor protein             | APP    | P05067-1            | 94.385  | 0.75       | 0.067       | Interaction Possible |
| Bone marrow stromal cell antigen 1              | BST1   | Q10588-1            | 88.2213 | 0.65       | 0.083       | Interaction Possible |
| Bone morphogenetic protein receptor, type II    | BMPR2  | Q13873-1            | 76.283  | 0.8        | 0.146       | Interaction Possible |
| Bruton agammaglobulinemia tyrosine kinase       | BTK    | Q06187-1            | 84.8525 | 0.75       | 0.377       | Interaction Possible |
| Casein kinase 2, alpha 1 polypeptide            | CSNK2A1| P68400-1            | 74.3077 | 0.65       | 0.653       | Interaction Possible |
| Caveolin-1                                      | CAV1   | Q03135-1            | 77.9051 | 0.6        | 0.138       | Interaction Possible |
| Caveolin-2                                      | CAV2   | P51636-1            | 83.1315 | 0.6        | 0.219       | Interaction Possible |
| CD59 molecule                                   | CD59   | P13987-1            | 82.6124 | 0.7        | 0.087       | Interaction Possible |
| Cysteine-rich, angiogenic inducer, 61           | CYR61  | Q06622-1            | 89.9187 | 0.7        | 0.424       | Interaction Possible |
| DLC1 Rho gtpase activating protein             | DLC1   | Q96QB1-2            | 78.2561 | 0.85       | 0.205       | Interaction Possible |
| Erb-b2 receptor tyrosine kinase 4*             | ERBB4  | Q15303-1            | 88.6078 | 0.8        | 0.22        | Interaction Possible |
| Estrogen receptor 1                            | ESR1   | P03372-1            | 82.6917 | 0.65       | 0.164       | Interaction Possible |
| Gap junction alpha-1 protein                   | GJA1   | P17302-1            | 71.9378 | 0.7        | 0.378       | Interaction Possible |
| Heat shock 70kda protein 5                     | HSPA5  | P11202-1            | 87.4218 | 0.65       | 0.163       | Interaction Possible |
| Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein | ID1 | P41134-1 | 63.6619 | 0.5 | 0.133 | Interaction Possible |
| Integrin, beta 1                               | ITGB1  | P05556-1            | 91.4368 | 0.8        | 0.086       | Interaction Possible |
| Integrin, beta 8                               | ITGB8  | P26012-1            | 91.3547 | 0.8        | 0.155       | Interaction Possible |
| Nitric oxide synthase trafficking              | NOSTRIN| Q8IV91-1            | 89.4255 | 0.65       | 0.063       | Interaction Possible |
| Phospholipase A2-activating protein             | PLAA   | Q9Y263-1            | 74.5552 | 0.7        | 0.199       | Interaction Possible |
| Platelet-derived growth factor receptor, alpha polypeptide | PDGFR | P16234-1 | 77.8343 | 0.8 | 0.176 | Interaction Possible |
| Polymerase I and transcript release factor     | PTRF   | Q6NZI2-1            | 78.2171 | 0.7        | 0.201       | Interaction Possible |
| Prostaglandin-endoperoxide synthase 2          | PTGS2  | P35354-1            | 86.235  | 0.95       | 0.232       | Interaction Possible |
| Protein C receptor, endothelial                | PROC7  | Q9UNN8-1            | 88.6295 | 0.75       | 0.1         | Interaction Possible |
| Protein disulfide-isomerase A3                 | PDIA3  | P30101-1            | 90.1417 | 0.85       | 0.583       | Interaction Possible |

(Continued)
Numerous studies have identified biomarkers and molecular signatures aimed at improving the diagnosis of mature T-cell lymphomas, herein we aimed to investigate shared molecular underpinnings that can be attributed to all subtypes. Specifically, our study brings novel insights into shared gene expression patterns (and confirmed protein expression of biopsy samples) amongst a diverse group of mature T-cell lymphomas. Our analysis uncovered 21 genes that shared differential expression in comparison to healthy CD4+ and CD8+ T cells. Together, these genes can be described as contributing to an activated phenotype, while suppressing differentiation into mature, effector T cells. Additionally, shared methods of apoptotic avoidance and ablations in TCR-related expression were observed.

Table 5. (Continued)

| Protein                                                                 | Symbol  | UniProt Sequence ID | IncPro^1 | RPISeq-RF^2 | RPISeq-SVM^3 | RPI-Pred         |
|-------------------------------------------------------------------------|---------|---------------------|----------|-------------|---------------|------------------|
| Protein kinase, camp-dependent, regulatory, type II, alpha              | PRKAR2A | P13861-1            | 93.0229  | 0.8         | 0.318         | Interaction Possible |
| Protein phosphatase 1, catalytic subunit, gamma isozyme                 | PPP1CC  | P36873-1            | 63.2618  | 0.6         | 0.387         | Interaction Possible |
| Protein tyrosine phosphatase, non-receptor type 11                       | PTPN11  | Q06124-1            | 78.559   | 0.6         | 0.022         | Interaction Possible |
| Ras homolog family member A                                             | RHOA    | P61586-1            | 66.3465  | 0.7         | 0.179         | Interaction Possible |
| RAS p21 protein activator                                               | RASA1   | P20936-1            | 73.3972  | 0.65        | 0.263         | Interaction Possible |
| Scavenger receptor class B, member 2                                     | SCARB2  | Q14108-1            | 77.3416  | 0.6         | 0.086         | Interaction Possible |
| Serum deprivation response                                              | SDPR    | O95810-1            | 73.1867  | 0.5         | 0.104         | Interaction Possible |
| SMAD family member 5                                                     | SMAD5   | Q99717-1            | 77.3416  | 0.75        | 0.096         | Interaction Possible |
| SMAD family member 7                                                     | SMAD7   | O15105-1            | 75.7177  | 0.65        | 0.239         | Interaction Possible |
| SMAD family member 9                                                     | SMAD9   | O15198-1            | 75.7465  | 0.6         | 0.147         | Interaction Possible |
| Son of sevenless homolog 1                                              | SOS1    | Q07889-1            | 91.8128  | 0.7         | 0.255         | Interaction Possible |
| Sterol carrier protein 2                                                 | SCP2    | P22307-1            | 72.2967  | 0.75        | 0.071         | Interaction Possible |
| Striatin, calmodulin binding protein                                    | STRN    | O43815-1            | 75.5192  | 0.65        | 0.065         | Interaction Possible |
| Transmembrane emp24 domain trafficking protein 2                        | TMED2   | Q15363-1            | 78.6391  | 0.8         | 0.327         | Interaction Possible |
| Transmembrane emp24-like trafficking protein 10                         | TMED10  | P49755-1            | 89.2447  | 0.65        | 0.522         | Interaction Possible |
| Tumor necrosis factor receptor superfamily member 6                     | FAS     | P25445-1            | 92.6536  | 0.6         | 0.091         | Interaction Possible |
| Wiskott-Aldrich syndrome-like                                           | WASL    | O00401-1            | 83.887   | 0.55        | 0.228         | Interaction Possible |

* Proteins in the CAV1-Low interaction network
^1 Interactions scored positive at values ≥50
^2 Interactions scored positive at values ≥0.5
^3 Interactions scored positive at values ≥0.5

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Discussion

Numerous studies have identified biomarkers and molecular signatures aimed at improving the diagnosis of mature T-cell lymphomas, herein we aimed to investigate shared molecular underpinnings that can be attributed to all subtypes. Specifically, our study brings novel insights into shared gene expression patterns (and confirmed protein expression of biopsy samples) amongst a diverse group of mature T-cell lymphomas. Our analysis uncovered 21 genes that shared differential expression in comparison to healthy CD4+ and CD8+ T cells. Together, these genes can be described as contributing to an activated phenotype, while suppressing differentiation into mature, effector T cells. Additionally, shared methods of apoptotic avoidance and ablations in TCR-related expression were observed.
Regarding a shared activated phenotype for T-cell lymphomas, of the observed upregulated genes, two (CAV1 and ENAH) have been implicated in cytoskeletal remodeling (and therefore, cellular migration and immune synapse formation) following TCR-ligation [15, 26]. Knockout studies of CAV1 that investigated immune synapse formation showed disruption of the actin cytoskeleton [15] and deregulation of cell membrane polarity [27]. These studies indicated an impairment of APC:T-cell engagement, and demonstrated a fundamental role for CAV1 as a co-stimulatory molecule in immune synapse formation, though the precise mechanism remains elusive. However, the upregulation of both CAV1 and ENAH could indicate an enhancement of T-cell stimulation and activation. This observation is strengthened by a pan-upregulation of TNFRSF21 (also known as DR6) and CCNB2. TNFRSF21 is a death domain-containing receptor of the tumor necrosis factor-receptor family [28] shown to be upregulated in peripheral CD4+ and CD8+ T cells following TCR-mediated T-cell activation in a time and NF-kB/NFAT dependent manner [29]. CCNB2 is a G2/M specific cyclin kinetically shown to be constantly upregulated throughout T cell stimulation [30].

Furthermore, the down regulation of TSC22D3 (also known as GILZ), TNFRSF14 (also known as HVEM), CD5, and UBASH3A (also known as STS-2) are in accordance with an activated phenotype. GILZ (glucocorticoid-induced leucine zipper) is an NF-κB inhibitor that prevents NF-κB binding and is independent from Rel- or IκB-related proteins [31]. Additionally, GILZ expression has been shown to be inversely correlated with T-cell activation [31, 32]. HVEM is down-regulated following T-cell activation, in concurrence with its description as a negative regulator of T-cell activation. HVEM+/− mice displayed a hyper responsive phenotype of enhanced proliferation and cytokine production (IFN-γ/IL-2) [33–35], a phenotype mirrored in STS-2−/− T cells [36]. CD5 has a demonstrated role in TCR-signaling wherein it acts as a negative regulator through an immunoreceptor tyrosine-based inhibitory motif (ITIM)-like motif [37]. The absence of CD5 in thymocytes induces a hyper-responsive state following TCR-stimulation [38]. CD5 expression is routinely examined during histological staining of T-cell lymphomas, and frequently harbors aberrant expression [13, 39, 40].

While displaying gene expression in accordance with an activated phenotype, all mature T-cell lymphoma samples additionally differentially regulated genes involved in effector T-cell differentiation (GILZ, TNFRSF21, STAT5A, RICTOR), T-cell homeostasis (FOXP1 and HVEM), and upregulating an immature thymocyte marker (Thy-1/CD90) [41]. The upregulation of TNFRSF21 and downregulation of GILZ, STAT5A, and RICTOR suggests a subversion of Th2-effector differentiation as T cells displaying similar phenotypes harbored impairment of Th2-cytokine production or population homeostasis [42–45]. This observation suggests a potential skewing of the neoplastic T cells towards other T-cell subsets or keeping them in an undifferentiated state. For example, the cellular origin of AITL has been well studied and established as follicular helper T cells [46–48]. Despite the established plasticity among helper T-cell subsets, inter-subset regulation (i.e. maturation into one effector subset blocking the machinery required for differentiation into other subsets) occurs regularly, and has been well described [49]. Taken together, these data suggests that mature T-cell lymphomas display gene expression characteristics that subvert certain aspects of the Th2 effector phenotype. This indicates that Th2 effector T cells may not represent a widespread cell-of-origin attributable to the mature T-cell lymphoma subtypes analyzed. However, recent studies have shown that upregulation of GATA3, the master transcription factor for Th2 effector T cells, is associated with a poor prognosis [8, 50], demonstrating that a small subgroup of malignant Th2 cells exist, representing a more aggressive disease phenotype.

Both FOXP1 and HVEM were found to be down-regulated in our analysis, and represent a disruption of T-cell maturation and the homeostasis of mature, effector T cells. FOXP1 is a member of the Forkhead box transcription factor family. FOXP1-deficient T cells display a
premature activation phenotype in the thymus and the acquisition of effector functions. This indicates an improper generation of quiescent, naïve T-cells [51, 52]. Further, HVEN-deficient mice displayed a defect in the homeostasis of the long-term memory T-cell population and this defect is independent of either Th1/Th2 populations [35]. Histological evaluation of FOXP1 in T-cell lymphomas has demonstrated that higher FOXP1 expression leads to an improved overall survival in PTCL patients and may be associated with a less activated tumor phenotype [53]. However, FOXP1 staining has proven to have disparate results [54, 55] and not all T-cell lymphomas have been found to be immunoreactive to FOXP1 [55].

Thy-1 (CD90), a marker found to be upregulated in all mature T-cell lymphoma subtypes, is a marker for thymus-derived lymphocytes in mice [56], with additional implications for being a “cancer stem cell” marker present in both T- and B-malignancies due to its upregulation on immature cells [41, 57, 58]. Additionally, CD90 has been described as a consistent marker in different adult stem cell populations, reviewed in [59]. This observation is further supported by inverse relationships noted between Thy-1 expression and T-cell differentiation or loss of stem-cell like potential [41, 60], with T-cell expression of Thy-1 restricted to a small population of cortical thymocytes in humans, in addition to staining in T cells surrounding germinal centers [61]. In our immunohistochemical analysis, 46% (29/65) of T-cell lymphoma samples scored positive for CD90 lymphoid staining, with the staining being light and membranous. These results are in agreement with the above-discussed immature/undifferentiated phenotype observed in mature T-cell lymphomas, and suggest that these tumors may also increase the amount of immature T-cells present within the lymph node microenvironment.

Down-regulations are observed for many genes involved in TCR-signaling and the proximal events associated thereafter (CD3G, ITK, LAT, PAG1, PDE4B, PRKCQ). The attenuation of TCR/CD3 complex protein expression and proximal signaling molecules is featured as a hallmark of ALCL [62–64] with similarities noted in CD30+ PTCL-NOS cases [55]. These demarcations were inferred from a lack of immunoreactivity in PTCL tissue samples. Here, we have shown a down regulation of the mRNA of the aforementioned genes can be attributed to all subtypes of mature T-cell lymphomas studied, but with markedly lower expression for some genes in ALCL cases (Table 2). Further, ablations in TCR expression are seen with the down-regulation of RICTOR, the scaffolding molecule for mTORC2 signaling. mTORC2 signaling is vital for TCR surface expression, wherein RICTOR-deficient thymocytes displayed attenuated TCR surface expression [65]. The dysregulation of the mTORC2 pathway is further validated by the observed down-regulation of TCR components (TRAC, TRBC1) and stabilizing molecules (TRAT1) seen across multiple T-cell lymphoma subtypes (S1 Table).

For genes involving in apoptotic resistance and immune evasion, we observed a down-regulation of IL23A, and upregulation of PSEN2. IL23 is an immunomodulatory cytokine with controversial findings in terms of its anti-tumor properties [66, 67]. Despite this, IL23 has been described as effective in its activity against B-cell malignancies both in vitro and in vivo [68, 69]. Down-regulation of IL23A by T-cell malignancies represents a potential mechanism of immune evasion, which is further supported by an observed down-regulation of IL12RB1 (seen in all subtypes except AITL, S1 Table), a component of the IL23 receptor [70]. PSEN2 is upregulated during some forms of apoptosis [71, 72], however its cleavage by caspase-3 (upregulated in all but AITL, S1 Table) results in a peptide fragment starting a negative feedback loop and inhibiting apoptosis [73]. These mechanisms of apoptotic avoidance represent potential therapeutic targets for further studies as α-IL23 has demonstrated activity against B-cell malignancies.

Using the top-scoring pairs method [18], the expression differential between CAV1 and BCL10 identified lymphoma samples with 98.4% sensitivity and 88.5% specificity. While the selection of CAV1 and BCL10 by the TSP method was a priori, the available genes were selected
due to their differential expression, introducing bias. However, the CAV1/BCL10 ratio is biologically relevant, with a prior inverse correlation observed in CAV1−/− T-lymphocytes [74], and BCL10 being downstream of CD26 signaling, of which CAV1 is the cognate ligand [75]. BCL10 has been prior described as down-regulated among T-cell lymphomas, an observation in accordance with our findings. Despite this, the exact role for BCL10 is still inconclusive [39, 76, 77]. Our results demonstrate interplay between CAV1 and BCL10 in T-cell lymphomas and warrants further investigation.

In our analyses, CAV1 was upregulated in each T-cell lymphoma subtype. Since CAV1 is linked to disease progression and metastasis in different malignancies [15, 78–83], we sought to investigate the role of CAV1 within the tumor microenvironment. PANTHER gene list analysis of CAV1-High versus CAV1-Low malignancies revealed an enrichment of multiple GO annotations for cytokine/chemokine activity, immune processes, and migration/motility within the CAV1-High group. The existence of the heterogeneity of CAV1 expression in T-cell lymphomas was further solidified with our immunohistochemical results, with 55% (36/65) of tumor biopsies harboring lymphoid staining of CAV1. Taken together, these data suggest that T-cell malignancies with higher CAV1 expression correlate with a GEP of possessing a more inflammatory tumor microenvironment and may hold more metastatic potential [78, 82, 83]. Further support for this is seen in the CAV1-protein networks determined by STRING. These networks displayed enrichment in proteins involved in cell migration and proliferation, indicating that CAV1 is directly involved in the aforementioned signaling processes and is an integral protein driving the differences within the CAV1-High and CAV1-Low tumor microenvironments. However, further analysis is required for confirmation of a role for CAV1 in disease progression in T-cell lymphomas. Chiefly, detailed research involving patient records and clinical characteristics is needed for a definitive conclusion. Such a study would be in-line with the current knowledge of CAV1 expression within malignant entities, and is warranted based on the abovementioned results.

Interestingly, we also observed a long non-coding RNA (LINC00273) to be one of the highest genes differentially expressed between the CAV1-High and CAV1-Low groups. As long non-coding RNA signatures have been previously shown to be predictive in cancer [84–87], we chose to investigate whether this long non-coding RNA harbored any potential interactions with CAV1, given that CAV1 was the basis upon which sample stratification was conducted. Surprisingly, using computational prediction methods, we show that LINC00273 is predicted to interact with CAV1 and members of a CAV1-interaction network in 3 out of 4 prediction metrics. The large discrepancy regarding RPISeq results could stem from the RPISeq-SVM having a lower accuracy in H. Sapiens non-coding RNA-protein interaction prediction (57.3%) as compared to RPISeq-RF (74.7%) [24]. While we observe a greater inconsistency in our data regarding prediction, both IncPro and RIPred achieved a consensus prediction with the more accurate RPISeq-RF. Protein-RNA networks have been demonstrated as indispensable for cellular homeostasis [88–90], and the further investigation of long non-coding RNA signatures in T-cell lymphomas might hold predictive or therapeutic implications.

Herein, we present novel work concerning shared molecular underpinnings of five subtypes of mature T-cell lymphomas. These results enhance the knowledge of the basic biology of T-cell lymphomas, leading to insights concerning their activation and differentiation states, while also uncovering novel markers for future study. Finally, we characterize a role for CAV1 within the tumor microenvironment, wherein higher CAV1 expression among mature T-cell lymphomas coincides with a more inflammatory and invasive gene expression profile. Future study investigating the clinical implications of CAV1 expression in mature T-cell lymphomas is warranted.
Materials and Methods

Ethics statement

All patient samples used within this study were from publicly available databases (i.e. gene expression samples) or commercial vendors (i.e. histology samples). Any and all patient disclosures were conducted within the original studies from which these samples were taken. The authors of this study state that they have neither tracing nor identification information that would disclose the identity of the patient samples herein.

Data collation and normalization

Raw (.CEL) files were downloaded from the NCBI Gene Expression Omnibus (GEO) and loaded into BRB-ArrayTools using MAS5.0 normalization [91]. All samples were from publicly available studies, as described in Table 1. In total, 52 healthy T-cell samples including: CD4+ T cells and CD8+ T cells taken from peripheral blood donors, and 187 non-treated, lymph node or splenic T-cell lymphoma biopsy samples were analyzed. All samples collated were prepared using HG-U133 plus 2 arrays. Replicate spots within arrays were averaged and multiple probes/probe sets were reduced to one per gene symbol using the maximally expressed probe (set) as measured by average intensity across arrays. Cell line samples, samples from non-lymphoid tissue, and samples not of interest to this study (e.g. B-cell samples and reactive tonsil samples) from the aforementioned datasets were discarded.

T-cell compartment signature

To investigate the T-cell compartments of the malignant lymphoma samples, a “T-cell compartment signature” was created to be used in analyses of differential expression, thereby helping to reduce expression signatures from stromal components generated by processing whole tumor biopsies. This signature was compiled from GO annotation sets for T-cell receptor signaling, T-cell co-stimulation, T-cell homeostasis, and T-cell differentiation (http://geneontology.org/). Upon merging annotation lists, excluding duplicate genes, and manual curation for genes not expressed in T cells, 217 unique genes met these criteria (S4 Table).

T-cell compartment differential expression analysis

For analysis of the differentially expressed genes between the T-cell compartment of malignant samples and healthy controls, the class comparison method among groups of arrays was used with the Multivariate Permutations test computed based on 1,000 random permutations. Genes were filtered according to the compiled T-cell compartment signature (see T-Cell Compartment Signature above) and results were deemed significant at p < 0.001, with a false discovery rate (FDR) of < 0.001. Each malignant subtype was analyzed separately followed by manual analysis of shared differentially expressed genes between each subtype as compared to healthy controls. Manual compilation allowed for the construction of a shared list of differentially expressed genes shared across each subtype.

Establishment of a CAV1-expression threshold

To stratify malignant samples that overexpress CAV1, all samples were analyzed for CAV1 expression via the 212097_at probe signal. A CAV1 expression threshold was calculated by taking two standard deviations above the mean of the CAV1 expression signal in healthy samples. Ninety-two percent (92%; 48/52) of healthy samples were below the calculated threshold, comprising 100% of non-outlier samples (identified as dots above the upper fence on the box-and-whisker plot). This threshold was selected as an adaptation of the 68-95-99.7 standard confidence interval.
deviation short hand. While the healthy CAV1 expression range did not follow a normal distribution, two standard deviations from the mean covered the abovementioned 92% of samples and the remaining 7% comprised outliers. This threshold was deemed acceptable as it classified all non-outlier samples within the healthy range and moving the threshold to three standard deviations exceeded all values within the healthy population. Malignant samples that were above the expression threshold were categorized as “CAV1-High”, while malignant samples below the expression threshold were categorized as “CAV1-Low”.

CAV1-High versus CAV1-Low differential expression analysis

For analysis of the tumor microenvironment of CAV1-High and CAV1-Low samples, only genes with a significant (p < 0.01) correlation (“R” cut-off ± 0.22) to CAV1 expression were included (n = 7,170) to reduce inter-malignancy variation. Differentially expressed genes (p < 0.001) were then determined using the class comparison among groups of arrays method with the Multivariate Permutations test computed based on 1,000 random permutations and a restriction on the proportion of false discoveries set at 1% with a confidence level of 99%.

Gene list analysis of CAV1-High vs CAV1-Low

Fold-change values for genes found to be differentially expressed between the CAV1-High and CAV1-Low groups were input into the PANTHER gene analysis tool (PANTHER version 9.0; http://pantherdb.org/) [19, 20]. PANTHER enrichment and overrepresentation (excluding genes downregulated in CAV1-High) analyses were performed using the Homo sapiens reference list. Annotation data sets utilized included: GO molecular function, biological process, cellular component (complete versions), PANTHER protein class, and PANTHER pathways. Results were deemed significant at p < 0.05 using the Bonferroni correction method.

CAV1-interaction networks

Genes upregulated in the CAV1-High and CAV1-Low expression groups were input separately into STRING v10 [92] to identify potential CAV1 protein-protein interaction networks. Interaction network constituents were determined by visual analysis of proteins shown to have direct or indirect interaction with CAV1. Enrichment analyses were conducted using the genome background and Bonferroni correction with results deemed significant at p < 0.001.

Long non-coding RNA-protein interaction prediction

Long non-coding RNA-protein interaction (RPI) was predicted using three separate programs: IncPro [23], RPISeq [24], and RPI-Pred [25]. As RPISeq offers two prediction methods, a total of 4 distinct prediction outcomes were computed. RPI prediction software inputs were compiled according to program specificity. For IncPro, FASTA amino acid sequences for CAV1-interacting proteins, in addition to the FASTA nucleotide sequence for LINC00273, were input into the IncPro web-based platform (http://bioinfo bjmu edu.cn/Incpro/). The same protocol was used for the RPISeq web-based platform (http://pridb gdcb iastate edu/RPISeq/). Equivocal information is required for the RPI-Pred platform (http://ctsb is wfubmc edu/projects/rpi pred/), in addition to protein block (PB) information [93]. As not all structures have been experimentally solved for the proteins of interest, the kPRED method [94] of PB structure prediction was used identical to Suresh et al. [25]. Per the literature, IncPro predictions were deemed positive for interaction scores ≥ 50, RPISeq for interaction scores ≥ 0.5, and RPI-Pred supplied text-based interpretations.
Tissue microarray immunohistochemical staining

LY6161 high-density lymphoma and normal lymph node tissue arrays were ordered from U.S. Biomax, Inc and stained using the following protocol. Tissue array slides were deparaffinized and hydrated through xylene and a graded alcohol series, rinsed for 5 minutes in water, and incubated for 5 minutes in 3% H₂O₂. Slides were then washed 2 times followed by antigen unmasking using 1x antigen unmasking solution (Vector Laboratories) for 30 minutes. Slides were then allowed to cool, washed 3 times in 0.1% Tween-20 PBS buffer, and blocked for 30 minutes with 2.5% normal horse blocking serum (Vector Laboratories). Slides were then stained with antibodies directed against BCL10 (1:100), CD90 (1:10), CAV1 (1:100), and GILZ (1:50), overnight at 4°C. Slides were subsequently washed 3 times, incubated for 30 minutes with ImmPRESS™ reagent (Vector Laboratories), and washed an additional 3 times. Staining was revealed in peroxidase substrate DAB solution, with rinsing in tap water. Slides were counterstained with Hematoxylin QS (Vector Laboratories) and mounted with permanent mounting medium (Sigma).

Immunohistochemical staining analysis

Stained slides were scanned using an iScan Coreo Au whole slide brightfield slide scanner (Ventana) and visualized using Ventana Image Viewer. Four independent researchers scored the slides based on the presence or absence of staining in lymphoid cells and/or the presence or absence of staining in stromal/non-lymphoid cells.

Supporting Information

S1 Fig. PANTHER enrichment analysis of the integrin signaling pathway. Molecule colors represent fold-change values (CAV1-High vs CAV1-Low): (dark blue = -3.361E00 to -1.536E00); (light blue = -1.536 to 1.542); (green = 1.542 to 1.715); (yellow = 1.715 to 1.882); (orange = 1.882 to 2.117); (red = 2.117 to 5.099).

S2 Fig. PANTHER overrepresentation analysis for the PANTHER pathway: inflammation mediated by chemokine and cytokine signaling. Genes upregulated in CAV1-High samples as opposed to CAV1-Low are represented in red, while genes in the reference list are represented in gray.

S3 Fig. PANTHER overrepresentation analysis for the PANTHER pathway: angiogenesis. Genes upregulated in CAV1-High samples as opposed to CAV1-Low are represented in red, while genes in the reference list are represented in gray.

S1 Table. Genes significantly up- or down-regulated in at least 3/5 of T-cell lymphoma subtypes.

S2 Table. Top-scoring pairs classification results.

S3 Table. Detailed analysis of immunohistochemical staining on individual TMA samples.

S4 Table. Genes comprising the T-cell compartment.
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Author Contributions

Conceived and designed the experiments: TAH CEC. Performed the experiments: TAH. Analyzed the data: TAH TDS HNS CEC. Wrote the paper: TAH TDS HNS CEC.

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