Long non-coding RNAs (lncRNAs) represent a novel class of functional RNA molecules with an important emerging role in cancer. To elucidate their potential pathogenetic role in chronic lymphocytic leukemia (CLL), a biologically and clinically heterogeneous neoplasia, we investigated lncRNAs expression in a prospective series of 217 early-stage Binet A CLL patients and 26 different subpopulations of normal B-cells, through a custom annotation pipeline of microarray data. Our study identified a 24-lncRNA-signature specifically deregulated in CLL compared with the normal B-cell counterpart. Importantly, this classifier was validated on an independent data set of CLL samples. Belonging to the lncRNA signature characterizing distinct molecular CLL subgroups, we identified lncRNAs recurrently associated with adverse prognostic markers, such as unmutated IGHV status, CD38 expression, 11q and 17p deletions, and NOTCH1 mutations. In addition, correlation analyses predicted a putative lncRNAs interplay with genes and miRNAs expression. Finally, we generated a 2-lncRNA independent risk model, based on lnc-IRF2-3 and lnc-KIAA1755-4 expression, able to distinguish three different prognostic groups in our series of early-stage patients. Overall, our study provides an important resource for future studies on the functions of lncRNAs in CLL, and contributes to the discovery of novel molecular markers with clinical relevance associated with the disease.

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Peripheral blood mononuclear cells from CLL patients and healthy donors, and B-cell populations from tonsils (i.e. naïve B-cells (N), marginal zone (MZ)-like, germinal center (GC), switched memory (M) B-cells), tonsillar and bone marrow plasma cells were obtained as previously described.\textsuperscript{26} Highly enriched CD19\(^+\) B-cells were characterized for IGHV mutational status, CD38 and ZAP70 expressions, NOTCH1 mutations, and cytogenetic alterations, that is, deletion of chromosome 13q (del13q), 11q (del11q), 17p (del17p) and trisomy chromosome 12 (12+), as previously described.\textsuperscript{25–29}

**RESULTS**

LncRNA expression in CLL samples. The lncRNAs expression profiling of leukemic cells from a cohort of 217 patients with early stage, Binet A CLL were investigated (Supplementary Table S1)\textsuperscript{25} and compared with those of different types of normal B-cells. These samples included six peripheral B-cell (pBC), four GC, three N, two MZ, three M, four tonsillar plasma cells and four bone marrow plasma cells.

To detect lncRNA expression, we applied a custom pipeline able to re-annotate the probes included in the Gene 1.0 ST expression array according to the LNCipedia annotation database. Such a strategy allowed us to investigate the expression levels of 1852 well-annotated and specific human lncRNAs, as previously reported.\textsuperscript{26}

Firstly, we searched for the B-cell subset sharing lncRNA expression similarities with CLL cells. Clustering analysis of the 26 normal B-cell subtypes was run on the 141 lncRNAs varying at least 1.5-fold in expression levels from the mean across the data set. Distinct lncRNAs profiles were associated with tonsillar plasma cells, bone marrow plasma cells, pBCs and GC cells, whereas M, MZ and N B-cells presented undistinguishable expression profiles and were considered as part of a single cluster (Figure 1a). Among these five B-cells clusters, multiclass SAM analysis identified 226 differentially expressed lncRNAs (Supplementary Figure S1) that were subsequently used to compare normal B-cell subsets with CLL samples. The principal component analysis analysis revealed that CLL were more similar to tonsillar MZ, M and N B-cells than to GC cells, pBCs, tonsillar plasma cells or bone marrow plasma cells (Figure 1b). This finding is similar to that reported by our previous study in the context of miRNA and small nucleolar RNA (snoRNAs) expression in the same CLL data set.\textsuperscript{36} Given the similarities of the lncRNA profile among CLL cells, tonsillar MZ, M and N B-cells as a whole were chosen as the ‘comparator’ B-cells to unravel lncRNA expression alterations in CLL. The classifier was first trained on an unbiased randomly selected cohort of 109 CLL cases (training set), and then applied to a validation set including the 108 remaining samples (Supplementary Table S1). The minimum error corresponded to 24 discriminant lncRNAs expression profiles between CLLs and comparator B-cells, leading to a good classification accuracy when applied to the validation patient set (overall: 99.14%; specificity: 100%; sensitivity: 99.07%) (Figure 1c).

Specifically, the 14 lncRNAs that were downregulated in CLL included Inc-TOMM7-1 (showing the lowest expression ratio CLL/comparator) and 8 lncRNAs that are located in chromosomal regions, such as 14q32, 2p and 22q, coding for the highly variable portions of the immunoglobulin genes or for IGHV pseudogenes. The lncRNAs classifier included 10 lncRNAs that were upregulated in CLL samples, among which Inc-SNX29P2-3 and Inc-SEL1L3-6 (see below).

Importantly, the 24-lncRNA classifier signature was validated on a publicly available independent cohort including 9 CLL and 10 normal samples, profiled on the same array.\textsuperscript{37} Specifically, we verified the predictive power of the 24-lncRNA model using leave-one-out and linear discriminant analysis as the cross-validation procedure and prediction method, respectively, and demonstrated its capability to discriminate robustly CLL from normal B-cell samples (overall accuracy: 100%, Supplementary Figure S2).

LncRNA expression in different CLL prognostic subgroups

CLL patients can be stratified into different prognostic groups based upon the presence/absence of cellular, molecular or cytogenetic markers. Here, we aimed at identifying specific lncRNAs signatures associated with each of these CLL subgroups (specified in Supplementary Table S1). We compared lncRNA expression of IGHV mutated (M-CLL, \(n = 131\)) vs unmutated (UM-CLL, \(n = 85\)) patients. The analysis identified 30 differentially
expressed lncRNAs (Supplementary Table S2). To reduce biases possibly due to group heterogeneity (i.e., the distribution of cytogenetic alterations within the two IGHV classes), we restricted the analysis to a homogeneous CLL subgroup with del13q as common and sole abnormality, including 31 UM and 73 M-CLLs. This approach led to the identification of 12 differentially expressed lncRNAs (Table 1), 10 of which were already detected in the comparison of unselected M- and U-CLL (Supplementary Table S2). In particular, we identified an upregulation of the lnc-IRF2-3 mapping at 4q35 and lnc-AC004696.1-1, which is located antisense, head to head, to ZNF667 gene at 19q13.

Overall, 12 and 20 lncRNAs distinguished CLL stratified based on ZAP70 or CD38 expression levels, respectively (Supplementary Tables S3 and S4). Among them, five lncRNAs (Inc-IRF2-3, Inc-AC004696.1-1, Inc-TNFRSF13B-5, Inc-C1orf132-1 and Inc-BACH1-1) were common to those identified in the above analysis (Supplementary Figure S3), which is in line with the notion that CD38 and ZAP70 represent in part surrogate markers of IGHV mutational status.

To identify lncRNA expression patterns characterizing the major cytogenetic aberrations, all the FISH 'negative' CLLs were considered as normal counterpart (78 patients). In addition, when del13q occurred with another cytogenetic abnormality, the latter was considered predominant for group inclusion. The results of each analysis are reported in Supplementary Tables S5–S8, while the most significant lncRNAs (top 10 score) are reported in Table 1. In detail, del13q patients showed an upregulation of lnc-SEL1L3-1, lnc-AMZ1-6, lnc-DTNB-2 and lnc-SNX29P2-3 (Supplementary Table S5), which were also downregulated in the comparator normal B-cells with respect to CLL samples (Figure 1c), and the downregulation of lnc-SPRYD7-1, also known as DLEU2. In addition, CLLs with del13q showed the downregulation of lnc-LIPG-3. This lncRNA has three different transcripts, including the independent transcriptional unit SCARNA17 and the non-coding RNA SNHG22, in the intron of which the SCARNA17 is located. Lnc-LIPG-3 is also downregulated in CLL samples with del11q and 12+, both of which showed an upregulation of lnc-AC004696.1-1. Only two lncRNAs were differentially expressed in patients with del17p. In particular, we found the downregulation of Inc-LTB3P-2 located at 11q13 antisense to both NEAT1 and MALAT1, the latter being a well-known putative oncogenic lncRNA found significantly correlated with Inc-LTB3P-2 expression in our CLL data set ($R = 0.54$, $P < 2.2e-16$).

Finally, we investigated the expression of lncRNAs specifically associated with the presence of NOTCH1 mutations (Supplementary Table S9). Again, among the seven lncRNAs differentially expressed, NOTCH1 mutated CLLs showed an upregulation of Inc-IRF2-3, Inc-AC004696.1-1 and Inc-BACH1-1,
and a downregulation of lnc-C1orf132-1, all of which were found to be deregulated in CLL subgroups with adverse prognostic factors.

LncRNAs interplay with genes and miRNAs in CLL

To gain evidence of lncRNAs that might influence gene expression, we evaluated the correlation between the expression level of the 1852 lncRNAs and that of the 17 788 coding transcripts unambiguously detected by the arrays. To be confident of predicting lncRNA–gene relationship, we focused on an lncRNAs/mRNAs ratio with a correlation coefficient \( r > 0.9 \) (Table 2), and found three distinct lncRNAs: (i) lnc-AC004696.1-1 (see above) which positively correlated with its antisense overlapping gene ZNF667, suggesting a potential \textit{in cis} regulation; (ii) lnc-NDST3-1, downregulated in del13q and del11q CLLs (Table 1), which positively correlated with the expression of RPL18A; and (iii) lnc-cYorf15A.1-2 that highly correlated with the expression of four genes located in sense or antisense orientation on chromosome Y about 7 Mb apart, and with two other genes, RPS4Y1 and ZFY, located on the short arm of chromosome Y. Since lnc-cYorf15A.1-2 and its correlated genes are expressed by males only, the correlation coefficients were recalculated in male patients and found to be below 0.8 for all of the six lncRNA–gene couples.

The relationship between lncRNAs and miRNAs also was studied in all patients of the cohort. To test the transcriptional relationships, the correlations between the expression levels of any lncRNA and miRNA were computed first. We identified 861 lncRNA-miRNA couples significantly anti-correlated in our database (\( q\)-value < 0.01). Then, one of the most common target
prediction algorithms (RNA-22) was run on each significantly correlated pair, using the miRNA sequences annotated on miRBase v20 and the LNCipedia IncRNA sequences corresponding to the fragments investigated by the probes on the array. Hence, we identified 11 IncRNA-miRNA anti-correlated couples supported by miRNA target prediction (q-value < 0.05; Table 3). Notably, we found the couples miR-331-3p/lnc-LIPG-3 (lnc-LIPG-3 downregulated with clinical relevance).

Identification of an IncRNA transcriptional profile with clinical relevance

Since the cohort analyzed was recruited in a perspective study on Binet A patients, we could investigate correlations between IncRNA profiles and PFS. Clinical data were available for 209/217 CLL. To this aim, a global test was run on the 471 lncRNAs the expression of which varied mostly over the data set. Eight IncRNAs showed significant association with PFS (P < 0.001; Supplementary Table S10) and five had a significant predictive value for PFS in univariate analysis (Table 4). The expression levels of Inc-IRF2-3, Inc-AC004696.1-1, Inc-C1orf132-1 and KIAA1755-4 were validated by qRT-PCR, showing a good concordance (Supplementary Figure S4 and Supplementary Table S11). Notably, Inc-KIAA1755-4 corresponds to the SNORA71A that is processed from the intronic region of the non-coding RNA SNHG17 (Supplementary Figure S4d).

Next, our analysis focused on Inc-IRF2-3 that had the highest predictive value for PFS by univariate test and was highly expressed in UM-CLLs. Interestingly, the highest Inc-IRF2-3 expression level was found in the subset of UM-CLL patients with the shortest PFS (Figure 2a). However, its predictive power lost strength (P = 0.051) after adjustment for covariate confounders, particularly CD38, likely because high expression levels of CD38 and Inc-IRF2-3 define largely overlapping CLL subgroups (Supplementary Figure S3). In order to improve the robustness of the IncRNAs as PFS predictors, Inc-IRF2-3 was combined with a second IncRNA from the group with the significant predictive value (Table 4). We evaluated each of these IncRNAs in combination with Inc-IRF2-3, based on the following scheme defining groups as (i) high/high, (ii) low/low or (iii) discordant expression levels. Among all the possible combinations tested, the couple Inc-IRF2-3 and Inc-KIAA1755-4 determined the best predictive model. Specifically, a better PFS corresponded to patients with a concomitant low expression of both IncRNAs (a ‘low-risk’ group, including 122 patients, 56%), whereas a worse PFS was associated with their concomitant high expression (‘high-risk’ group, 12 patients; Figure 2b). The ‘intermediate-risk’ group (75 patients) was characterized by the discordant expression of the two IncRNAs. The high-risk group had a hazard ratio of 8.05 (95% CI: 3.82–16.96; median PFS: 862 days), and the intermediate-risk group of 2.27 (95% CI: 1.39–3.69; median PFS: 1678 days), compared with the low-risk group (median PFS not reached). Finally, multivariate regression analysis confirmed the independence of the 2-IncRNA risk model from other known predictive factors in CLL (IGHV mutational status, CD38 and ZAP70 expression, NOTCH mutation and unfavorable chromosomal aberration as covariates; Table 5). Likewise, the 2-IncRNA risk model resulted informative when compared with the recently defined progression-risk score (PRS) based on the integration of

| Table 3. LncRNAs highly correlated with miRNAs |
|------------------------------------------------|
| IncRNA | miRNA | IncRNA Chr. | IncRNA aliasa | Correlation | Target prediction |
|--------|-------|-------------|---------------|-------------|-------------------|
|        |       |             |               | cor | q-val | IncRNA target sequence from/to | q-val |
| C1orf86-1:2 | mir-221-3p | 1p36 | AS to PRKCaZ | -0.27 | 0.007 | (668, 690) | 0.011 |
| MAP1L3C8B-2:4 | mir-30b-3p | 12q24 | Linc00173 | -0.27 | 0.007 | (1890, 1911) | 0.045 |
| LIPG-3:2 | mir-331-3p | 18q21 | SNHG22, SCARNA17 | -0.26 | 0.008 | (2485, 2506) | 0.013 |
| MON2-2:2 | mir-370-3p | 12q14 | S to miR-lent7i | -0.27 | 0.007 | (10737, 10759) | 0.019 |
| TFDLP2-7:3 | mir-486-3p | 3q23 | GKS | -0.37 | 3.2E-05 | (3303, 3321) | 0.040 |
| LINS-1:2 | mir-574-3p | 15q26 | PRKX1 | -0.28 | 0.004 | (5285, 5306) | < 1E-06 |
| DKL1-4:12 | mir-628-3p | 14q32 | S to MEG3 | -0.26 | 0.010 | (4377, 4397) | 0.043 |
| C22ORF32-1:6 | mir-659-3p | 22q13 | Linc-OGRF1P1 | -0.29 | 0.003 | (2093, 2092) | 0.023 |
| SEC61G-1:12:1 | mir-659-3p | 7p12 | RN7SKP218 | -0.27 | 0.008 | (98, 120) | 0.047 |
| KIDINS220-6:14 | mir-92a-1-5p | 2p25 | Linc00298 | -0.28 | 0.004 | (184, 206) | 0.011 |
| SNHG15-1:8 | mir-940 | 7p13 | SNHG15 | -0.35 | 1.0E-04 | (2090, 2113) | 0.049 |

Chromosomal localization (Chr.) and alias name are indicated. *Sense (S) to, or antisense (AS) to, overlapping transcripts.

| Table 4. LncRNAs with significant predictive value related to PFS in the univariate log-rank test |
|-----------------------------------------------|
| IncRNA | Alias/overlapping transcript | Chr. | HR | LO 95% CI | UP 95% CI | P-valuea |
|--------|-----------------------------|-----|----|------------|-----------|--------|
| IRF2-3 | Linc | 4q35 | 3.67 | 2.14 | 6.28 | 2.14E-06 |
| C1orf132-1 | S to mir-102 | 1q32 | 0.36 | 0.22 | 0.59 | 5.49E-05 |
| ADAP2-2 | RN7SIL138P | 17q11 | 0.37 | 0.22 | 0.63 | 0.000245 |
| AC004696.1-1 | ZNF667-AS1 | 19q13 | 2.67 | 1.69 | 4.22 | 2.29E-05 |
| KIAA1755-4 | SNORA71A | 20q11 | 2.21 | 1.39 | 3.50 | 0.000691 |
| ZNF131-1 | Uncharacterized loc. | 5p11 | 2.07 | 1.31 | 3.25 | 0.0016 |
| PTPDC1-7 | AS to MIRLET7D | 9q22 | 0.45 | 0.28 | 0.74 | 0.00184 |
| MTRMR2-1 | Pseudogene | 11q21 | 0.53 | 0.33 | 0.84 | 0.00691 |

*aBased on the K-means clustering stratification of CLL cases into two groups according to IncRNAs expression level, five out of the eight IncRNAs had a significant predictive value for PFS in the univariate log-rank test (P < 0.001).
clinical, laboratory and biological parameters independently associated with PFS\(^3\) (Table 5).

To gain insight into their possible role in CLL pathophysiology, we evaluated in silico whether the modulation of lnc-IRF2-3 or lnc-KIAA1755-4 might correspond to transcriptional signatures possibly associated with functional categories. Specifically, we ranked genes according to decreasing Pearson’s correlation with the expression level of lnc-IRF2-3 or lnc-KIAA1755-4 in the 217 samples using the gene set enrichment analysis software to identify a priori-defined sets of genes showing concordant modulation. We found lnc-IRF2-3 associated with 18 gene sets (1 from Kegg and 17 from Hallmarks data sets; Supplementary Table S12; representative examples in Figure 3a), virtually all related to the metabolism of amino acids, sugars and lipids. Interestingly, lnc-IRF2-3 resulted associated with the gene set linked to primary immunodeficiency, and with genes encoding proteins over-represented on the apical surface of epithelial cells (Figure 3a). Concerning lnc-KIAA1755-4, we identified 30 gene sets (6 from Kegg and 24 from Reactome data sets; Figure 3b and Supplementary Table S13), 10 of which including genes mainly encoding for ribosomal proteins, and thus principally associated with ribosome formation and translational processes. Notably, 12 gene sets highly enriched in genes encoding for histone components are associated with transcriptional processes, chromosomes and telomeres maintenance, and telomeres packaging.

**DISCUSSION**

This study provided a comprehensive analysis of the transcriptional profile of lncRNAs in a large cohort of early-stage CLL patients.

First, we unraveled a 24-lncRNA signature specifically deregulated in CLL compared with a normal B-cell counterpart. The identification of the normal counterpart is currently a debated issue.\(^3\),\(^9\) Although based on a limited number of normal samples, our analysis revealed that the lncRNA expression profile of leukemic cells is more similar to N, MZ and M B-cells than to GC cells, total pBCs or PCs, as previously observed in our previous studies for miRNA and snoRNA expression patterns.\(^2\),\(^5\),\(^3\) Therefore, lncRNAs that significantly discriminate CLL cells and N, MZ and M B-cells, may be of relevance in disease pathogenesis. Importantly, the 24-lncRNA model was validated on an independent data set. Among the 24 lncRNAs, we highlighted the downregulation of lnc-TOMM7-1, mapped to chromosome 7p antisense to the interleukin-6 (IL6) gene, which promotes B-cell lineage proliferation and differentiation. Lnc-TOMM7-1 may participate in IL6 transcriptional regulation and therefore may have a pathogenic role, given the potential function of IL6 as an autocrine growth factor in CLL.\(^2\) The lncRNAs classifier also included lnc-SNX29P2-3

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**Table 5.** Multivariate analysis comparing the 2-lncRNA risk model with prognostic variables or with PRS in CLL series

| Variable     | HR   | LO 95% CI | UP 95% CI | P-value |
|--------------|------|-----------|-----------|---------|
| I-RISK GROUP | 2.32 | 1.30      | 4.12      | 0.0043  |
| H-RISK GROUP | 7.64 | 2.66      | 21.92     | 2E-04   |
| UM-CLL       | 1.38 | 0.65      | 2.92      | 0.396   |
| ZAP70+       | 0.84 | 0.43      | 1.60      | 0.596   |
| del17+       | 1.67 | 0.54      | 5.11      | 0.366   |
| del11+       | 3.98 | 1.72      | 9.19      | 0.001   |
| 12+          | 1.28 | 0.57      | 2.84      | 0.541   |
| CD38+        | 1.00 | 0.99      | 1.01      | 0.243   |
| NOTCH-MUT+   | 0.94 | 0.49      | 1.81      | 0.861   |
| I-RISK GROUP | 1.54 | 0.85      | 2.78      | 0.149   |
| H-RISK GROUP | 3.88 | 1.63      | 9.18      | 2E-03   |
| PRS          | 3.18 | 2.08      | 4.83      | 6.5E-08 |

*Intermediate-risk or high-risk group in the 2 lncRNA-risk model.
and lnc-SEL1L3-6 which were upregulated in CLL samples. This finding, contradictory to that found by us in PC dyscrasia where the two lncRNAs are downregulated in pathological samples, suggests that the role of lncRNA likely depends on the cellular context.

Despite the homogeneity of the lncRNA expression profile, possibly enhanced by testing samples of patients uniformly at their early disease stages, specific lncRNA signatures were detected in subgroups of CLL stratified according to cellular, molecular and cytogenetic markers. As expected, some lncRNAs were recurrently associated with the presence of adverse prognostic markers. Among them, it is worth mentioning lnc-AC004696.1-1, also known as ZNF667-AS1, the highest expression of which was found in UM-CLLs as also showed by previous studies, and was also negatively associated with a short PFS. Interestingly, two recent studies have reported epigenetic silencing of this lncRNA during the immortalization of human mammary epithelial cells as well as in a panel of cancer types including acute myeloid leukemia, diffuse large B-cell lymphoma and solid tumors. These findings seem contradictory to the situation that we and others have found in CLL with adverse prognosis for which high lnc-AC004696.1-1 expression was compatible with a transformed and potentially aggressive condition, again supporting the notion that the role played by lncRNA likely depends on the cellular context. Notably, in our CLL database, lnc-AC004696.1-1 expression highly correlated with that of which was found in UM-CLLs as also showed by previous studies, and was also negatively associated with a short PFS. Interestingly, two recent studies have reported epigenetic silencing of this lncRNA during the immortalization of human mammary epithelial cells as well as in a panel of cancer types including acute myeloid leukemia, diffuse large B-cell lymphoma and solid tumors. These findings seem contradictory to the situation that we and others have found in CLL with adverse prognosis for which high lnc-AC004696.1-1 expression was compatible with a transformed and potentially aggressive condition, again supporting the notion that the role played by lncRNA likely depends on the cellular context. Notably, in our CLL database, lnc-AC004696.1-1 expression highly correlated with that
of the ZNF667 gene (Table 2), with which it shares (head to head) a CpG island. A co-regulatory mechanism of the two transcripts needs to be investigated, but it is of interest the evidence that ZNF667 inhibits the expression of the anti-apoptotic gene BAX in rats.  

Albeit it is feasible that IncRNAs are engaged in miRNA mediated interactions, their contribution is yet poorly explored in general, and in CLL in particular. To provide insights into this aspect, we studied the expression correlation of IncRNAs and miRNAs in paired samples, with the support of target prediction analysis. Among the 11 pairs reported in Table 3, particularly relevant appear miR-574-3p/Inc-LINS-1 and miR-331-3p/Inc-LIPG-3. MiR-574-3p is upregulated in UML-CLL and is associated with a major risk of disease progression in the same cohort of CLL.  

Considering that miR-574-3p has been described as a tumor suppressor miRNA in different types of solid tumors, as we can hypothesize that in CLL, Inc-LINS-1 may play a fine-tuning role in the regulatory circuitry including miR-574-3p and its target genes. Considering the other miRNA/IncRNA pair, Inc-LIPG-3 expression was downregulated in samples with del13q, del11q or 12+ (Table 1). While existing data describe miR-331-3p as one of the most expressed miRNA in CLL as compared with normal peripheral CD19+ B-cells, our results do not highlight such a great difference with the normal comparators (Supplementary Figure S5) and is in all likelihood due to the different B-cell subpopulation chosen as normal controls. Notably, a network between miR-331-3p and IncRNAs has been already described in gastric cancer, where miR-331-3p has been proven to target the HOXAIR IncRNA that, in turn, functions as a competing endogenous RNA (ceRNA) to regulate HER2 expression by sponging miR-331-3p.  

Finally, we have proposed a 2-IncRNA risk model, based on the expression of Inc-IRF2-3 and Inc-KIAA1755-4, able to stratify our series of early-stage Binet A CLL patients into three different prognostic groups. The model identifies a very-high-risk group characterized by the concomitant high expression of both IncRNAs. Notably, the model is independent of the common prognostic markers and of a recently defined progression-risk score. Information on Inc-IRF2-3 is still very limited. Ferreira et al. reported its high expression in prognostically poor UM-patients (Supplementary Table S2). Conversely, Inc-IRF2-3 is progressively downregulated through the more aggressive stages of PC dyscrasia. Such a discrepancy maybe due to the cellular context as previously hypothesized for Inc-SNMX2P9P2-3 and Inc-SEL1L3-6. Our gene set enrichment analysis results pointed out that in CLL, Inc-IRF2-3 expression is associated with 18 gene sets of which 15 (15%) are related to the metabolism of amino acids, sugars and lipids. Among the remaining ones, it is of note the gene set linked to primary immunodeficiency given that it includes genes related to CLL biology such as Zap70, CD19, BTK and CD79A genes. In addition, we found a specific association between Inc-IRF2-3 expression and the gene set that included genes related the apical surface of epithelial cells, such as those important for determining cell polarity. Overall these data prompt to further investigation of the functional role of Inc-IRF2-3 in the biology and the progression of the disease. Lnc-KIAA1755-4 is processed from the intronic region of the non-coding RNA SNHG17 and actually corresponds to SNORA71A, thus belonging to a class of molecules essentially localized in the nucleolus where they function as guide RNAs for the post-transcriptional modification of ribosomal RNAs. In particular, SNORA71A is predicted to guide the pseudouridylation of U406 in 18S rRNA. Functional analysis of genes whose expression might be related to Inc-KIAA1755-4 highlighted enrichments in genes encoding for histone components, genes associated with transcriptional processes, chromosome and telomere maintenance, and telomeres packaging, all of which are biological processes frequently affected during cellular immortalization and tumor progression.  

Overall, our findings offer a portrait of the IncRNA transcriptional landscape in CLL, ultimately providing insights into the biological mechanisms involving the non-coding fraction of the transcriptome and contributing to suggest novel putative molecular markers associated with high-risk course of the disease.  

CONFLICT OF INTEREST  

The authors declare no conflict of interest.  

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