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# Long Non-coding RNAs Including IRF1-AS1, LINC01871, TRG-AS1, and USP30-AS as Tumor Suppressors in Colorectal Cancer: In Silico Analysis

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## Abstract

**Introduction:** Colorectal cancer (CRC) is one of the most frequently diagnosed malignancies in the world with a high mortality rate, making screening and early detection of the condition essential. Long non-coding RNAs (lncRNAs) have a significant role in the initiation and advancement of numerous malignancies, including CRC, by taking part in the control of gene and protein expression that affects apoptosis, cell proliferation, and immunological responses. In this study, through bioinformatics methods, we investigated this non-coding group in CRC and the normal group in both early and advanced stages of the disease.

**Materials and Methods:** In order to identify the lncRNAs that could have a tumor-suppressing role in CRC, RNA sequencing data from the cancer genome atlas (TCGA) were analyzed. Then, the Pearson correlation test was applied between the expression level of candidate lncRNAs and all genes expressed for identifying potential pathway. Genes with the highest correlation were selected and subjected to gene enrichment analysis. Also, the roles of identified lncRNAs were evaluated in terms of biomarkers.

**Results:** The results of the expression analysis for the TCGA data showed that the expression of the IRF1-AS1, LINC01871, TRG-AS1, and USP30-AS decreased during the progression (stages III and IV compared with stages I and II) of CRC. The enrichment results of all the genes in the co-expression network related to IRF1-AS1, LINC01871, TRG-AS1, and USP30-AS showed that these lncRNAs could play a role in immune response, inflammation, and IL-6/JAK/STAT3 signaling and apoptosis pathways. Additionally, our findings demonstrated that the aforesaid lncRNAs were significantly lower in CRC samples compared with normal samples based on TCGA data, Also, the expression of some of them may serve as an appropriate biomarker.

**Conclusion:** The results of this study showed that IRF1-AS1, LINC01871, TRG-AS1, and USP30-AS decreased during the progression of CRC and could play a tumor-suppressing role.

**Keywords:** Biomarkers, Colorectal cancer, Long non-coding RNAs, Immune response.

## 1. Introduction

The prominent gastrointestinal tumors are colorectal cancers (CRC), esophageal cancers and gastric cancers [1]. Statistics show that CRC is the third most common and diagnosed cancer in the world and fourth in cancer related death [2]. The CRC incident is higher in developed countries with Western culture. A number of etiological elements can be associated with the development of CRC, including

environmental, genetic and epigenetic [3]. Most CRCs arise from adenomatous polyps stemming from the glandular epithelium of the intestine. Adenomas are caused by somatic mutations in the tumor suppressor gene APC regulator of WNT signaling pathway. Additional genetic alterations in oncogenes and tumor suppressor genes are involved in a stepwise growth process over years. Accumulation of genetic mutations consistent with chromosomal instability transforms normal intestinal mucosa into adenomatous polyps, then high-grade adenomas, and finally carcinomas. CRC can also arise from non-polyposis and depressive degeneration [4]. The risk of CRC death increases due to metastasis to other organs of the body [5]. Therefore, there is an urgent need to explain the molecular mechanisms that underlie CRC development and uncover new markers and therapeutic strategies.

Nearly 70% of genome transcripts are not translated into any protein and form non-coding genes, which are divided into short and long categories based on their length [6]. Short non-coding RNAs are often recognized as negative regulators of gene expression, while long non-coding RNA (lncRNA) is still poorly understood [7]. Most of them are more than 200 nucleotides in length and lack open reading frame [6]. Based on the involvement of non-coding RNAs in cancer, their key role can be pointed out. Many studies have found out about their role in the spread of various cancers and their irregular expression in tumorigenesis. Therefore, they can be considered as tumor suppressors or oncogenes [6]. GAS5 is a tumor suppressor lncRNA that decreases in many cancers, including CRC, breast, lung, and prostate [8]. A recent study found enhanced expression of lncRNA-APC1 by directly binding to Rab5b mRNA; it inhibits cell proliferation, angiogenesis, and metastasis of CRC, thereby suppressing exosome production. lncRNA-APC1 expression is controlled by APC via her PPARα accumulation on the lncRNA-APC1 promoter [9]. These findings and others indicate that lncRNAs participate in a number of cellular functions and can contribute to the pathogenesis of CRC.

The function and role of many lncRNAs are still unknown, despite the fact that their involvement in critical cellular processes and the pathogenesis of diseases such as CRC has been demonstrated. The objective of this study was to find lncRNAs that can act as tumor suppressors and that exhibit a reduction in expression as CRC progresses. LncRNAs that decrease in advanced stages compared to early stages were found using the cancer genome atlas (TCGA) data. The pathways connected to the discovered lncRNAs were then assessed. Additionally, their differences in expression from normal samples were looked into.

## 2. Materials and Methods

### Data collection and analysis

In order to identify the lncRNAs involved in the progression of the disease having a significant decrease in expression, the RNAseq data available in the TCGA database was used through deploying the TCGAbiolinks package for CRC. Removal of genes with zero or close to zero expression with counts-per-million (CPM) criterion less than 10 in 50% of samples was done using the edgeR package. In the following, normalization of data based on a trimmed mean of M values (TMM) method and transfer of data to the Log2 scale were done by the limma package. The obtained expression profile was used to do the analyses. Also, the latest update of clinical data for CRC was downloaded and used to divide samples and correlate gene expression with patient survival. In general, TCGA data included 41 normal samples, 268 samples for early stages (stage I, II) and 199 samples for advanced stages (stage III, VI). Also, the list and specifications of all lncRNAs were downloaded from the HUGO database.

### Differential lncRNA expression and biomarker analysis

In order to investigate the expression changes of lncRNAs in advanced stages (AS), early stages (ES) and normal samples, their expression differences were calculated through the linear model method. For this purpose, expression matrix data were normalized and used in a Log2 scale, and the list of all lncRNAs extracted from the HUGO site was examined in the mentioned groups. Also, receiver operating characteristic (ROC) chart was used to investigate the expression changes of candidate lncRNAs as diagnostic biomarkers.

### Co-expression network and enrichment analysis

A co-expression network was constructed in order to identify pathways related to candidate lncRNAs. At first, Pearson's correlation test was applied between the expression levels of candidate lncRNAs and all genes expressed in TCGA colon cancer data. Then, the genes that had the highest expression correlation with candidate lncRNAs ($R>0.5$ and $P<0.01$) were selected. Next, co-expression network was constructed using the Cytoscape tool. To identify pathways related to co-expression network, data enrichment was done using Enrichr tool and MsigDB repository (https://maayanlab.cloud/Enrichr/).

### Statistical analyses

All pre-processing and data analyses were done by R software (version 4.0.2); GraphPad Prism software (version 9.0) was used to display graphs. The linear model method was used to calculate the difference in expression, and the significance level between the groups was calculated through multiple hypothesis testing. The FDR<0.05 level was considered in all analyses. Cytoscape software (version 4) was deployed to show the co-expression network and the relationship between genes and lncRNAs.

## 3. Results

### Decreased expression of IRF1-AS1, KBTBD11-OT1, LINC00324, LINC01871, TRG-AS1 and USP30-AS1 during CRC progression

TCGA data for CRC were used to identify the lncRNAs that can decrease during disease progression and play a tumor suppressor role. In the first step, the expression difference between ES samples compared with AS was calculated and the results showed that 26 lncRNAs significantly decreased in AS compared with ES, whose results are summarized in Table 1 (Adj. P.Value<0.05).

**Table 1.** 26 lncRNAs that decrease in AS samples compared to ES are shown.

| lncRNA names | Adj.P.value | logFC (AS vs ES) | lncRNA names | Adj.P.value | logFC (AS vs ES) |
|---|---|---|---|---|---|
| TNFRSF10A-AS1 | <0.01 | -0.39 | LINC01871 | <0.01 | -0.45 |
| USP30-AS1 | <0.01 | -0.42 | PINK1-AS | <0.01 | -0.51 |
| KLRK1-AS1 | <0.01 | -0.31 | KLHDC7B-DT | <0.01 | -0.36 |
| LINC02195 | <0.01 | -0.35 | LINC02446 | <0.01 | -0.33 |
| LINC00114 | <0.01 | -0.32 | TRG-AS1 | <0.01 | -0.29 |
| SNHG3 | <0.01 | -0.27 | DLG3-AS1 | <0.05 | -0.28 |
| LNCTAM34A | <0.05 | -0.25 | DANCR | <0.05 | -0.24 |
| IRF1-AS1 | <0.05 | -0.23 | TFAP2A-AS1 | <0.05 | -0.21 |
| IQCH-AS1 | <0.05 | -0.25 | KBTBD11-OT1 | <0.05 | -0.24 |
| LINC02605 | <0.05 | -0.21 | EP300-AS1 | <0.05 | -0.31 |
| ZNF232-AS1 | <0.05 | -0.27 | RGMB-AS1 | <0.05 | -0.41 |
| SNHG16 | <0.05 | -0.37 | LINC00324 | <0.05 | -0.37 |
| LINC02100 | <0.05 | -0.35 | CLLU1 | <0.05 | -0.26 |

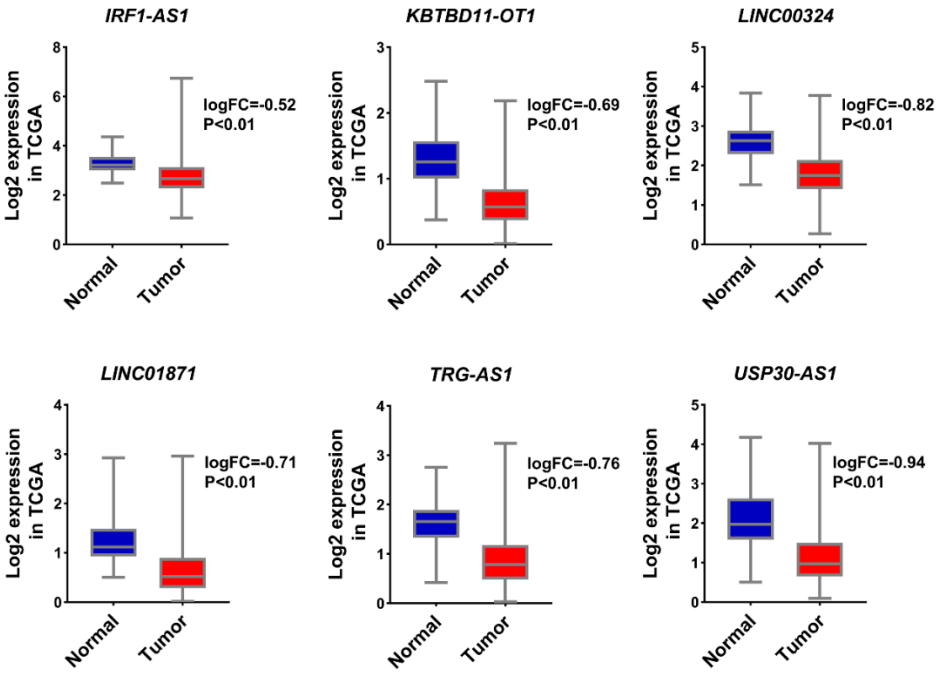


**Figure 1.** The expression of candidate lncRNAs reduced in cancer samples compared to normal. The expression level of candidate lncRNAs in tumor samples compared to normal is shown based on TCGA data. The normalized matrix was used in logarithmic scale to draw graphs and calculate the expression difference. (logFC = log fold change, P = Adj.P.Value).

Next, the expression levels of 26 lncRNAs identified in cancer samples were compared to normal and the results showed that only 6 of the 26 lncRNAs which included *IRF1-AS1*, *KBTBD11-OT1*, *LINC00324*, *LINC01871*, *TRG-AS1* and *USP30-AS1* decreased in cancer samples (Figure 1, LogFC<-0.5, FDR<0.01). These results show that the 6 mentioned lncRNAs decrease during the progression of CRC and can play a role in the malignant process.

## Expression level of KBTBD11-OT1 and TRG-AS1 as a diagnostic biomarker

In the following, the receiver operating characteristic (ROC) curve was used to investigate the changes in the expression of lncRNAs identified in the previous step as a diagnostic biomarker. The results showed that the expression changes of *KBTBD11-OT1* in cancer samples compared to normal can be a suitable diagnostic biomarker for patients with CRC (Figure 2, AUC>0.9, P<0.01). Also, the results for other candidate lncRNAs revealed that among them, the expression level of *TRG-AS1* could be used as a good diagnostic biomarker. These results indicate that the mentioned lncRNAs can be suggested as biomarkers in CRC.

## Inflammatory and apoptotic pathways associated with identified lncRNAs

Pathways related to the identified lncRNAs were analyzed through the co-expression network. The results of the correlation test showed that a total of 502 genes were significantly related to the identified lncRNAs (Figure 3A). Also, no genes were found for *KBTBD11-OT1* and *LINC00324*. Afterwards, 502 identified genes were enriched using Enrichr database. Enrichment results revealed that most of 502 genes are significantly involved in immune pathways such as Interferon Gamma Response, Complement, IL-6/JAK/STAT3 Signaling and Apoptosis (Figure 3B, FDR>0.01). These results suggest that *IRF1-AS1*, *LINC01871*, *TRG-AS1* and *USP30-AS1* could play a role in the pathogenesis of CRC through the regulation of pathways related to immunity and apoptosis.

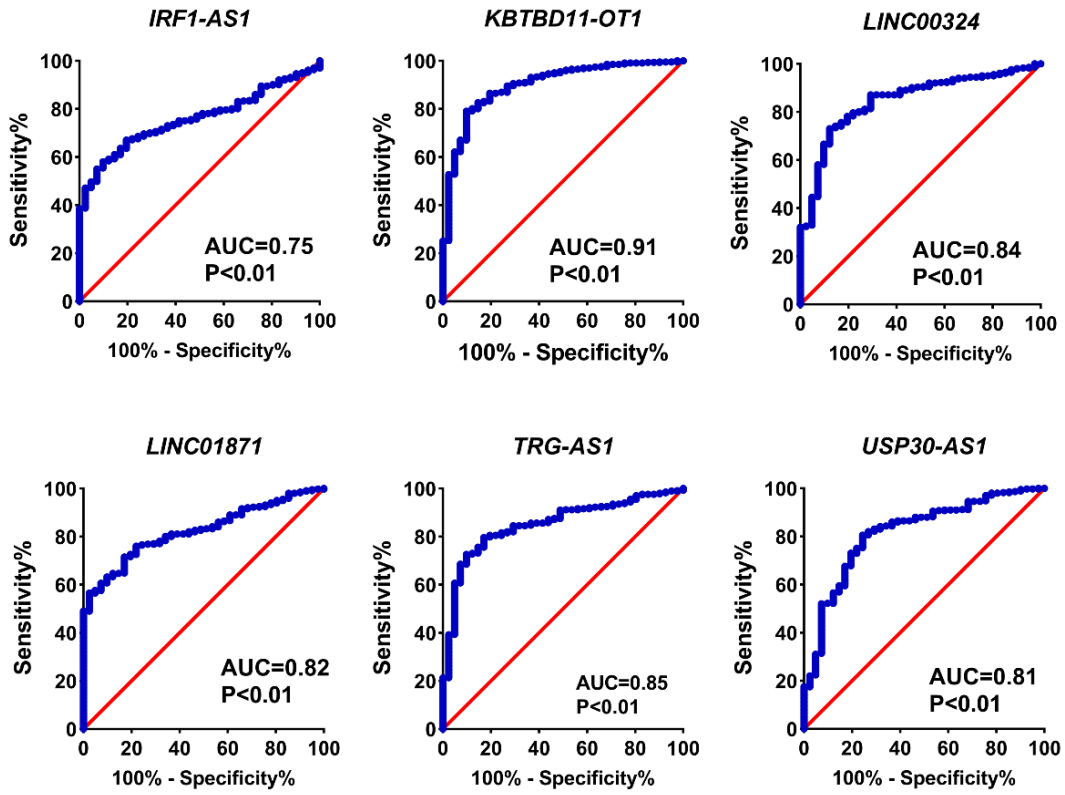


**Figure 2.** The expression level of *KBTBD11-OT1* and *TRG-AS1* can be used as a diagnostic biomarker in colorectal cancer. ROC plots for 6 candidate lncRNAs are shown. For ROC analysis, the expression level of each lncRNA in normal samples compared to cancer samples was used (AUC: Area under the curve).

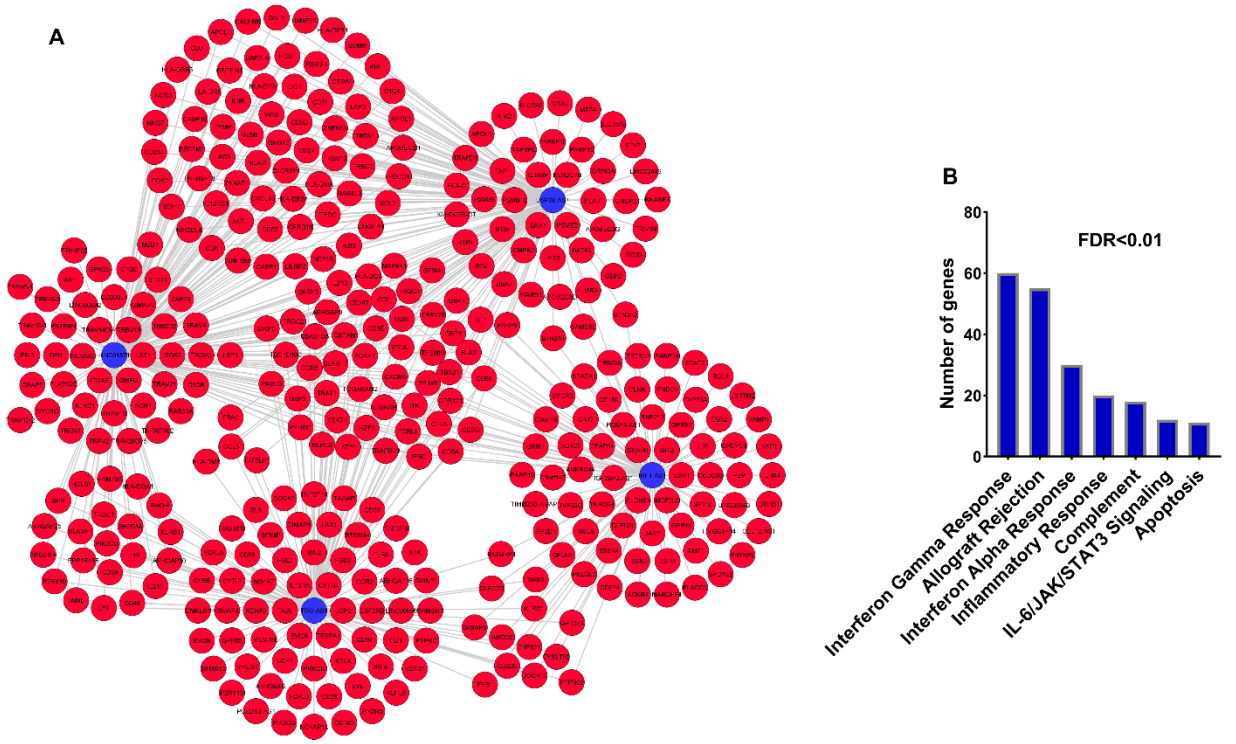


**Figure 3.** Expression association of candidate lncRNAs with genes related to immune and apoptosis pathways. (A) Co-expression network for all genes co-expressed with candidate lncRNAs. Genes were selected with criteria of R>0.5 and P<0.01 based on TCGA data. (B) Enrichment results for all genes in the co-expression network are shown.

## 4. Discussion

In this research, we investigated lncRNAs in CRC compared with those in the normal group and also investigated the AS of the disease compared with the ES. The difference in gene expression in the two ES and AS groups was determined with the help of TCGA data in order to find out which LncRNAs are involved in the more advanced stages of the disease and which are decisive in the progression of the disease. We found 6 key LncRNAs, all of which play a role in tumor suppression in CRC. One of these candidate LncRNAs, *IRF1-AS*, has already been shown to be a tumor suppressor in both in vivo and in vitro environments in esophageal squamous cell carcinoma [10]. Through the JAK-STAT pathway, interferons cause the production of *IRF1-AS*. In addition to the traditional JAK-STAT pathway, IFNs also activate the CRKL, NF-B, and MAPK pathways [11].

*LINC01871* was another of our identified candidates, which clearly plays a role in the activation of immune responses in breast cancer cells, especially the basal type. This type of LncRNAs has a strong correlation with immune response genes like *GZMB*, *CTLA4* and *PDCD1* [12]. Furthermore, in the TCGA gastric cancer cohort, the expression of *LINC01871* was favorably linked with CD8+ T cell enrichment levels, cytolytic immunological activity, and CD274 (PD-L1) expression levels [13]. *LINC01871* was also discovered in a predictive signature associated with immune response and the TGF- signaling pathway in cervical cancer [14]. Our study confirmed that *LINC01871* can be employed as a reliable prognostic marker for patients with CRC since its expression was linked to the activation of the Interferon Gamma response. This can also be assessed by focusing on the effectiveness of the therapy, primarily immune-therapy. The molecular mechanisms through which *LINC01871* is engaged in the process of immune system activation need to be understood further, in order for it to be fully relevant as a biomarker.

According to previous studies, lncRNA T cell receptor gamma locus antisense RNA 1 (*TRG-AS1*) is a potent driver of the oncogenicity of tongue squamous cell carcinoma through the regulation of the microRNA-543/Yes-associated protein 1 axis [15], promoting the proliferation of glioblastoma cells by competitively binding with miR-877-5p to regulate the expression of *SUZ12* [16]; it stimulates the progression of hepatocellular carcinoma by sponging miR-4500 to modulate BACH1 [17]. *TRG-AS1* was markedly overexpressed in samples of lung cancer. *TRG-AS1* was

upregulated, which aided in the proliferation and invasion of cancer cells. In addition, it was discovered that *TRG-AS1* was targeting miR-224-5p [18]. However, studies conducted in colorectal cancer have shown that *TRG-AS1* decreased in CRC and its increased expression inhibits proliferation and metastasis [19]. Additionally, our findings demonstrated that this *TRG-AS1* lowers as the disease worsens and has the potential to limit tumor growth.

Prior research has suggested that *USP30-AS1* mediates the development of a number of human cancers, including glioblastoma, cervical cancer, and acute myeloid leukemia [20-22]. Recently, *USP30-AS1* has been identified as one of the candidates involved in colon cancer. The prognosis and malignancy of patients with CRC are predicted by *USP30-AS1*. It has been made clear that altering miR-765, *USP30-AS1* prevented CRC from progressing [23]. Our findings also showed that the expression of *USP30-AS1* during CRC progression and also in cancer samples significantly reduced in comparison with normal.

ROC curve analyses showed that *KBTBD11-OT1* and *TRG-AS1* can separate malignant tissues from non-malignant ones with diagnostic power of 0.85. The other marker (Table 1), however, was unable to differentiate between samples that were malignant and those that were not. Also, through gene enrichment analysis, we examined the genes that had a significant correlation with these LncRNAs. It was found that these LncRNAs are related to key pathways related to cancers, such as “apoptosis”, “Allograft rejection”, “Interferon alpha”, “inflammatory” and “Interferon Gamma”. Finally, our findings showed that the expression of the mentioned lncRNAs decreases during the progression of the disease and can play a tumor suppressor role. These results, however, need to be confirmed by future ex vivo and in vitro studies; this is one of the major limitations of this study.

## 5. Conclusion

We have reported a number of related lncRNAs that are dysregulated in CRC, in summary. In the pathoetiology of CRC, the current investigation demonstrates a potential involvement for *IRF1-AS1*, *LINC01871*, *TRG-AS1*, and *USP30-AS1*. However, functional investigations are needed to identify the mechanisms underlying their contribution to this process.

## Ethical Considerations

### Compliance with ethical guidelines

Not Applicable.

### Funding

Not available.

### Author's contributions

The design and conceptualization of study and methodology was done by A.F.J. and K.G. Data mining, formal analysis and investigation was performed by A.F.J.; supervision, validation and visualization was done by H.N., M.F. and K.G. Interpretation of the obtained information was done by A.F.J. and K.G. The manuscript was written by A.F.J. Review, editing and approved by H.N. and K.G. All authors read and approved the final manuscript.

### Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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