A novel long non-coding RNA regulates the integrin, ITGA2 in breast cancer

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Abstract
Purpose ITGA2 encodes the integrin, α2 which mediates metastatic progression, and is a predictor of poor prognosis and chemoresistance in breast cancer. Decreased ITGA2 promoter methylation is implicated as a driver of increased gene expression in aggressive prostate and pancreatic tumours, however the contribution of altered methylation to ITGA2 expression changes in breast tumours has not been examined.

Methods ITGA2 gene methylation and gene expression was examined in publicly available breast cancer datasets, and ITGA2 promoter methylation was mapped by targeted bisulphite sequencing analysis in breast tumour cell lines. The expression of a putative regulatory long noncoding RNA (lncRNA) was examined by qPCR and its’ functionality was investigated using gene knockdown (antisense oligonucleotides) and over expression in breast cancer cell lines.

Results In breast tumours and breast cancer cell lines the ITGA2 promoter is largely unmethylated, with gene expression variable in tumour subtypes, irrespective of promoter methylation. A novel lncRNA (AC025180.1;ENSG00000249899), named herein I2ALR, was identified at the ITGA2 gene locus, and was variably expressed in breast tumours and breast cancer cell subtypes. I2LAR knockdown resulted in upregulation of ITGA2 gene expression, whilst over-expression of I2ALR resulted in downregulation of ITGA2 mRNA. Further, examination of two downstream targets of ITGA2 associated with breast tumor stemness and metastasis (CCND1 and ACLY), revealed concomitant gene expression changes in response to I2ALR modulation.

Conclusion I2ALR represents a novel regulatory molecule targeting ITGA2 expression in breast tumours; a finding of significant and topical interest to the development of therapeutics targeting this integrin.

Keyword lncRNA · Integrins · ITGA2 · Epigenetic · Gene regulation

Introduction

Cell surface molecules, including integrins, are critical for metastasis and invasion. Integrins are adhesion receptors with extracellular and cytoplasmic signalling domains. The integrin ITGA2, or α2 (CD49b), forms the α2β1 collagen type I (col-I) and laminin receptor [1, 2], a key mediator of metastasis and marker of poor prognosis in solid tumors including breast, ovarian and pancreatic cancers [3–6]. In normal breast tissue, α2β1 is expressed in the mammary gland at cell–cell/basement membrane interface [7], and is regulated by estrogen [8]. Primary breast cancer (BrCa) tumors retain the elevated α2β1 expression and the estrogen receptor positive (ER+) status of normal breast tissue, with expression of α2β1 and ER often lost in soft tissue metastases [4]. Luminal/ER+ BrCa cell lines (MCF-7, T-47D or MCF-10A) have been reported to express elevated ITGA2, relative to basal-metastatic/ER− lines (MDA-MB-231 and MDA-MB-436) [9–11]. However, MDA-MB-231 cells cultured on bone matrix highly express α2 relative to MCF-7 and T-47D cells [12], and preferentially metastasize to bone, where they express elevated a2 [13]. In addition, α2β1

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expression promotes stem-cell like behaviour in triple negative BrCa [14].

Epigenetic modifications facilitate the nuanced control of gene expression and play an important role in the regulation of integrins. DNA-methylation, histone modifications, micro RNAs and long non-coding RNAs (LncRNAs) co-ordinate to deliver precise expression of genes, and present exciting chemotherapeutic options. MiRNAs including miR-373-3p and miR-206 are reported to post-transcriptionally regulate ITGA2 protein levels in BrCa [11, 14]. The ITGA2 promoter includes a CpG island (CpGI) which in normal prostate tissues and pancreatic cancer is hypomethylated and associated with upregulated ITGA2 expression. Methylation at the promoter is increased in the locally invasive LNCaP PrCa cell line which expresses low levels of ITGA2. Whilst in bone metastatic PC3 cells, decreased promoter methylation is associated with high α2β1 expression [3, 15], which is also reflected in PrCa bone metastases [16]. Histone modifications also regulate ITGA2 [17], and it is hypothesised that a combination of these epigenetic mechanisms contribute to control of expression of this gene in different tumor tissue contexts.

In the present study, we demonstrate that methylation at the ITGA2 promoter CpGI in BrCa cells was not associated with changes in ITGA2 expression, in contrast to previous observations in PrCa, suggesting alternative regulatory mechanisms are at play. Examination of the ITGA2 promoter flanking regions revealed a previously uncharacterized lncRNA, herein named I2ALR (AC025180.1, ENSG00000249899, also referred to as lnc-MOCS2-1). This study provides insight into the tissue-specific differential epigenetic mechanisms regulating ITGA2 and provides important context to current efforts to develop ITGA2-targeted therapeutics.

Materials and methods

Cell culture

Cell lines employed for this study included the BrCa cell lines MDA-MB-231, MDA-MB-453, MCF-7 and T-47D and were obtained from the American Tissue Cell Collection (ATCC, USA) and the PrCa cell lines, PC3 and LNCaP were obtained from Cell Bank Australia (CMRI, Sydney). Cell passaging conditions are described in the Supplementary Methods including a description of cell line characteristics (Supplementary Methods Table 1). Cell lines were verified by DNA fingerprinting performed by the Australian Genome Research Facility (May 2021).

Quantification of gene expression

Cell pellets were obtained and RNA was extracted using the RNaseasy® Mini Kit (QIAGEN) and converted to cDNA using the iScript™ Reverse Transcription Supermix kit (Bio-Rad Laboratories Inc.). Alternatively, SuperScript® III First-Strand Synthesis Kit (Invitrogen, Thermo Fisher Scientific) was used to specifically detect poly-A transcripts, using an oligo(dT)20 primer, and RNase H. Expression levels of genes of interest were quantitated using quantitative PCR and levels are expressed relative to the housekeeping gene GAPDH. Primer sequences are presented in Supplementary Methods Table 2. Analyses were performed in triplicate using SensiFAST™ SYBR® No-Rox (Bioline). Thermal cycling conditions are presented in Supplementary Methods Table 2. Standard curves were generated to determine absolute gene expression. Gel electrophoresed PCR products were extracted with QIAquick® Gel Extraction Kit (QIAGEN®).

Methylation analysis of ITGA2 CpGI

To map CpGI methylation at the ITGA2 promoter, gDNA was extracted from cell pellets using DNeasy Blood & Tissue kit (QIAGEN), and bisulphite converted with the EZ DNA Methylation-Gold™ Kit (ZYMO Research). Amplified regions of the bisulphite converted promoter (see Supplementary Methods Table 3 for primer sequences) were generated using MyTaq™ HS Mix (Bioline). Fragments were ligated with pGEM®-T Easy Vector System I (Promega) and transformed used SoloPack® Gold Competent Cells (Agilent Technologies). Ten bacterial colonies were selected per amplified region, cultured and DNA extracted with the QIAprep® Spin Miniprep Kit (QIAGEN). DNA sequence was obtained using an ABI 3500 Genetic Analyzer (Applied Biosystems), and analysed using Sequencher® software, v4.10.1 (Gene Codes Corporation), and converted into bubble maps (representing the CpG sites) using BiQ Analyzer software (V2.0, Max Planck Insitut fuer Informatik) and Microsoft EXCEL ‘CpG Bubble Chart Generator’ (V.20061209 alpha, Mark A. Miranda).

Knockdown of IncRNA I2ALR

Knockdown of IncRNA I2ALR in MDA-MB-453 cells was achieved using antisense oligonucleotide (ASO) gapmers. Two targeting ASOs and a scrambled control (ASO-Scr) were designed and synthesised by Integrated DNA Technologies (Supplementary Methods Table 4). ASOs were transfected into cell lines using the INTERFERin® in vitro siRNA/miRNA transfection reagent protocol (Polyplus-Transfection), at a final optimized ASO concentration of
10 nM (in 10.5 mL of media), with cells at ~50% confluency, and cultured for 24 and 48 h before harvesting.

**Overexpression of lncRNA I2ALR**

To over-express I2ALR, a construct, named I2ALR<sub>construct</sub>, was synthesised and cloned into pUC57-Kan by GENEWIZ Inc. (USA). The I2ALR<sub>construct</sub> contained the combined exons for ENST00000505701.5 and ENST00000503559.1, including an uncharacterized 3′ region (U3R) plus the poly-A signal, and 100 bps of polyadenylation following the poly-A signal (I2ALR<sub>construct</sub>, Supplementary Methods Table 5). Using NheI-HF and XhoI restriction enzyme sites incorporated at 5′ and 3′ flanks respectively, the I2ALR<sub>construct</sub> fragment was ligated into pcDNA<sub>TM</sub>3.1(+) mammalian expression vector (Invitrogen) and transformed into SoloPack Gold Competent *E. coli* cells. PCR screening using ‘I2ALR set 5′ primers (Supplementary Methods Table 2) identified positive colonies which were cultured, and plasmid isolated for subsequent transfection. MCF-7 cells were selected for over-expression experiments as they had moderate ITGA2 and I2ALR expression. Cell lines were transfected by electroporation (5.0 x 10<sup>8</sup> cells per cuvette + 10 µg of plasmid, voltage 230 V, 950 µF, ∞ Ω, distance 4.0 mm). Cells were harvested after 48 h.

**Publicly available datasets**

Data for ITGA2 mRNA expression and ITGA2 methylation were obtained from cbioPortal for Cancer Genomics (https://www.cbioportal.org/) [18]. Datasets: “Breast Invasive Carcinoma (TCGA, Cell 2015)” [19], “Metastatic Prostate Adenocarcinoma (SU2C/PCF Dream Team, PNAS 2019)” [21], “Prostate Adenocarcinoma (TCGA, Cell 2015)” [22].

**Bioinformatic analysis of the I2ALR lncRNA**

Prediction of lncRNA cellular localization used LncLocator (Shanghai Jiao Tong University, http://www.csbio.sjtu.edu.cn/bioinf/LncLocator/) [23]. Interactions between lncRNA and mRNA were predicted with IntaRNA 2.0 bioinformatics tool (V. 4.5.10, Freiburg RNA Tools, http://rna.informatik.uni-freiburg.de/IntaRNA/Inta.jsp) [24]. The secondary structure of lncRNA was predicted with RNAfold (v. 2.4.13, University of Vienna, http://rna.thi.uniwe.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) [25]. Analysis of overall and disease free survival used GEPIA (Zhang Lab. Peking University, http://gepia.cancer-pku.cn/), with comparison of high and low expression groups using Log-rank test (Mantel-Cox test) and high and low cohort group cut-off thresholds being quartile (cut-off high 75%, cut-off low 25%) [26].

**Statistical analysis**

Comparisons of the differences was conducted with Student’s t-tests or one-way ANOVA. Student’s t-tests were conducted for comparison of two groups or for when equal variance (homoscedasticity) was appropriate. One-way ANOVA analysis was conducted in Rstudio, with Tukey’s post-hoc testing on the ANOVA models. All reported p-values are two tailed.

**Results**

**ITGA2 expression is variable in BrCa and PrCa cell lines**

Expression of ITGA2 mRNA was examined in cell lines representing different subtypes of BrCa. Expression in MDA-MB-231 cells was 16-fold higher compared with the lowest expressing MDA-MB-453 cells. T-47D and MCF-7 cells displayed intermediate expression (Fig. 1A). Differential expression is also observed between PrCa cell lines with bone metastasis derived PC3 cells showing elevated ITGA2 expression relative to lymphnode metastasis derived LNCaP cells (Fig. 1A, with significant expression differences shown) (one-way ANOVA, Tukey’s post-hoc tests, P < 0.05).

Analyses of publicly available datasets revealed that reduced ITGA2 expression was a significant predictor of distant metastases (Student’s t-test, P < 0.05, Fig. 1B) in BrCa. Expression of ITGA2 in BrCa and PrCa was elevated in primary tumors and bone metastases and reduced in soft tissue metastases. Statistical significance was evident between the primary tumors and soft tissue metastases in BrCa datasets (Student’s t-test: P < 0.001, Fig. 1C; P < 0.01, Fig. 1D), although statistical comparisons were impacted for the BrCa bone metastasis by the small sample size available. In the PrCa dataset, ITGA2 expression differences between bone metastases compared to soft tissue metastases were significant (Student’s t-test, P < 0.01, Fig. 1E).

**DNA methylation of the ITGA2 promoter CpGIs**

Methylation mapping of the ITGA2 promoter in BrCa cell lines by bisulphite sequencing revealed hypomethylation irrespective of the level of ITGA2 expression (Fig. 2A). In the BrCa dataset (TCGA Cell 2015), ITGA2 promoter methylation was elevated in BrCa soft tissue metastases and lower in both bone metastases and primary tumors (Fig. 2B), although not statistically significant. ITGA2 promoter methylation was only weakly inversely associated with expression in the BrCa samples (TCGA Cell 2015) (Fig. 2C). However, a stronger correlation was observed in PrCa samples (TCGA...
Fig. 1 ITGA2 mRNA levels in BrCa, PrCa cell lines and publicly available tumor datasets. A Expression of ITGA2 in BrCa and PrCa cell lines shown as absolute copy number normalized to GAPDH, ±SEM (n = 3 biological replicates). B ITGA2 expression as predictor of distant BrCa metastasis, TCGA Firehouse Legacy data. C ITGA2 mRNA expression in primary tumors relative to bone and soft tissue metastases in BrCa, TCGA Cell 2015 data, D Firehouse Legacy data and E in PrCa, PNAS 2019 data. B–E mRNA expression = RNA-Seq-V2 RSEM values or FPKM capture values. P-value notation, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001
Fig. 2 ITGA2 promoter methylation in BrCa and PrCa. A From top: diagrammatic representation of the 600 bp ITGA2 promoter comprising a CpG of 46 CpG sites (vertical grey bars). Diagrammatic representation (bubble maps) of CpG sites, each line represents a cloned DNA fragment (5'–3'). Open and black circles represent unmethylated and methylated CpG sites, respectively. CpG sites 1–4 (left panel) and 5–46 (right panel). B−D ITGA2 methylation in BrCa and PrCa tumors. B Methylation of ITGA2 reported in the public dataset TCGA Cell 2015 for BrCa primary tumours and metastases. C ITGA2 mRNA expression versus ITGA2 methylation reported in BrCa dataset TCGA Cell 2015 (n=551 primary tumour samples). D ITGA2 mRNA expression versus ITGA2 methylation reported in the PrCa dataset TCGA Cell 2015 (n=289 primary tumour samples). C, D mRNA expression = RSEM values (RNA Seq V2 data). B−D methylation levels = β values
Cell 2015) (Fig. 2D). Interestingly, the majority of PrCa samples showed high overall ITGA2 promoter methylation (methylation β-scores > 0.6, and none with scores < 0.4). By comparison, promoter methylation varied across the full spectrum at the ITGA2 promoter in BrCa samples. Statistical analysis revealed these differences between BrCa and PrCa to be significant (Student’s t-test, P < 0.001). These data suggest that alternative regulatory mechanisms may be at play.

Expression of IncRNA I2ALR in BrCa and PrCa

In seeking to understand alternative mechanisms employed by BrCa and PrCa cells in the regulation of ITGA2, the gene locus was examined, revealing an uncharacterised IncRNA transcribed in reverse, downstream of the ITGA2 transcription start site. Given that IncRNAs are known to regulate genes in cis, we sought to examine whether this novel IncRNA regulated ITGA2. The uncharacterised IncRNA gene AC025180.1 (ENSG00000249899), was designated ITGA2 Antisense LncRNA (I2ALR). I2ALR expression assessed by qPCR was relatively low compared to ITGA2 expression, and of the examined BrCa cell lines, I2ALR was predominantly expressed by MDA-MB-453 cells, followed by T-47D and MCF-7, and MDA-MB-231 with the lowest expression. Expression in the PrCa cell lines PC3 and LNCaP is presented for comparison (Fig. 3A). In BrCa cell lines, I2ALR expression levels were weakly inversely correlated with levels of ITGA2 mRNA (Fig. 3B). In contrast, and somewhat surprisingly, in PrCa cell lines I2ALR expression was highest in PC3 cells (Fig. 3A) in which ITGA2 is also highly expressed (Fig. 1A).

Knockdown of I2ALR increases ITGA2 expression

To determine whether modulation of I2ALR influenced ITGA2 expression, antisense oligonucleotides (ASOs) were designed to target the IncRNA (Supplementary Results Fig. 1A). Transient transfection of two ASOs, ASO-160 and ASO-991, targeting regions of I2ALR, into MDA-MB-453 cells at increasing concentrations (10 nM, 25 nM and 50 nM) revealed both ASOs induced I2ALR knockdown relative to the ASO scrambled control (ASO-Scr) after 24-h. I2ALR knockdown was clearly evident at the lowest concentration (10 nM) and this concentration was therefore selected...
Fig. 4 I2ALR lncRNA knockdown (KD) and overexpression effects on ITGA2 and its downstream target genes. A, B MDA-MB-453 cells transfected with ASO-160 or ASO-991 targeting I2ALR, or a non-targeting ASO-Scrambled (ASO-Scr) control after 24-h (final ASO concentrations 10 nM, n = 3 biological replicates, untreated cells included as controls). I2ALR mRNA levels are shown in (A) and ITGA2 mRNA expression in (B). C, D MCF-7 cells transfected with the over-expression I2ALR construct, a pcDNA3.1 + vector control, or untreated (n = 3 biological replicates). C I2ALR overexpression in MCF-7 cells vs. controls, y-axis scale adjusted to log base 10. D ITGA2 expression in MCF-7 cells overexpressing I2ALR compared with vector only and untreated controls. E, F Expression of ACLY (E), and CCND1 (F) in MCF-7 cells overexpressing I2ALR versus vector only and untreated controls, 48 h following transfection. G, H Expression of ACLY (G) and CCND1 (H) in MDA-MB-453 cells transfected with ASO-160 at 10 nM inducing I2ALR knockdown, 48 h following transfection. RNA levels = absolute copy number normalized to GAPDH, ± SEM (n = 3). Statistical significance *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, one-way ANOVA Tukey’s post-hoc test used for all analyses, with the exception of ‘F’ which was significant for Student’s t-test only.
Characterisation of I2ALR

A diagrammatic representation of the I2ALR in relation to the ITGA2 promoter is shown in Fig. 5A showing two putative TSSs. The DNA sequence is shown in Fig. 5B, with additional analysis using alternative primer pairs revealing that the classical poly-A signal is the likely preferred motif to the alternative poly-A signal (see Supplementary Results Fig. 2 for additional analyses). Analysis of lncRNA localization using LncLocator predicts subcellular localizations based on lncRNA sequence. Analysis of the combined exons of ENST00000503559.1 and ENST00000505701.5 indicated that I2ALR was predicted to be translocated to the cytoplasm (57%), and inclusion of the uncharacterized 3′-UTR did not substantially alter this prediction (55%). The predicted localization of I2ALR was also consistent with experimental cell line data available in the LncExpDB database for this transcript (HSALNG0041756) [27].

Given that lncRNAs are known to form lncRNA-[RNA binding protein]-mRNA complexes, putative interactions were investigated in silico between the 1112 bp I2ALRmRNA sequence and ITGA2 mRNA using the IntaRNA 2.0 bioinformatics tool. Interactions with a seed sequence of > 6 ideal base-pairings were considered, yielding five energetically favourable pairings (Supplementary Results Fig. 3A). The lncRNA secondary structure was also predicted with RNAfold (Supplementary Results Fig. 3B), with two hairpin loop domains apparent and a minimum free energy of −319 kcal/mol.

I2ALR expression and cancer survival

To examine whether I2ALR is associated with clinical outcome, expression of I2ALR was examined in publicly available cancer datasets. Analysis of GEPIA data revealed elevated I2ALR expression (normalized to GAPDH) in BrCacases. A statistically significant decrease in ACLY expression was observed when compared with controls (P < 0.05, one-way ANOVA Tukey’s post-hoc test). A smaller decrease in CCND1 expression also observed (P < 0.05, Student’s t-test, although non-significant using one-way ANOVA) (Fig. 4E and F).

In a second breast cancer cell line, targeted KD of I2ALR by ASO-160 in MDA-MB-453 cells (associated with increased ITGA2 expression) was also observed to result in a concomitant increase in expression of ACLY and CCND1 (P < 0.05; one-way ANOVA, Tukey’s post-hoc test) (Fig. 4G and H) at 48 h. These results are consistent with ITGA2-dependent regulation of ACLY and CCND1 reported by Adorno-Cruz et al. [14].

Overexpression of the putative I2ALR transcript reduces ITGA2 expression

To further confirm an I2ALR regulatory effect on ITGA2, an I2ALR over-expression construct, I2ALRconstruct was transfected into MCF-7 cells. High expression of I2ALRconstruct was confirmed in transfected cells compared with the vector control and untreated controls (Fig. 4C; one-way ANOVA; Tukey’s post-hoc tests, P < 0.001). Expression of ITGA2 was reduced by over-expression of I2ALR relative to vector and un-transfected controls (Fig. 4D; one-way ANOVA, Tukey’s post-hoc tests, P < 0.001).

Effect of I2ALR on downstream ITGA2 targets

In order to determine whether I2ALR-mediated knockdown of ITGA2 impacted downstream genes, the expression of two known ITGA2-responsive genes, CCND1 and ACLY was examined. Increased ITGA2 expression upregulated these genes promoting stemness and metastatic progression in BrCa [14]. Both ACLY and CCND1 were down-regulated by over-expression of I2ALR in MCF-7 cells. A
of other tumor types revealed higher I2ALR expression correlated with improved overall survival (Fig. 5D and F).

Discussion

Several studies have now shown that multiple epigenetic mechanisms regulate ITGA2 expression in solid tumors including altered promoter methylation and post transcriptional regulation by miRNAs [11, 28]. In prostate tumors ITGA2 expression is highly correlated with promoter hypermethylation [15], however it was shown that in breast tumors the promoter is largely hypomethylated, yet ITGA2 expression remains highly variable. Similarly, BrCa cell lines exhibit hypomethylation at the ITGA2 promoter irrespective of expression, consistent with a recent report [29]. Here we describe a novel IncRNA (I2ALR) which is variably expressed in BrCa cell lines and functions in BrCa cells to regulate ITGA2 gene expression. Further we provide evidence that I2ALR activity may be cell-type specific, as its expression did not correlate with ITGA2 expression in prostate tumor cells.

Adorno-Cruz et al. [14] have recently reported ITGA2 expression is variable in breast tumours with high expression associated with reduced survival, and the significance and magnitude of this effect varies between BrCa sub-types. Here we accessed GEPIA data, and consistent with this finding these analyses showed that higher I2ALR expression was associated with improved survival, up to approximately 120 months, over a similar time frame as that reported by Adorno-Cruz et al. [14]. We were not able to examine I2ALR expression in breast tumor sub-types using the GEPIA data, as it did not permit subclassification based on breast tumor sub-type or stage. This may have impacted the ability detect a significant association with survival. More in depth examination of a role for this IncRNA in breast cancer sub-types is warranted. For I2ALR, other antisense IncRNAs also regulate expression of their adjacent gene; e.g. HOTTIP repressing HOXA13 [34], and BLNCR regulating ITGB1 [35]. I2ALR was found to comprise 5 exons, with a polyadenylated extended terminal exon (U3R). We hypothesise I2ALR forms a IncRNA-RNA binding protein-mRNA complex, destabilizing ITGA2 mRNA, in a similar manner to the IncRNAs, RP11 and HOXA11-AS. These IncRNAs bind cytoplasmic hnRNPA2B1 and STAU1 proteins, and mRNAs FBXO45/ SIAH1 and KLF2 respectively. The IncRNA-mRNA complementary interactions guide specificity in the case of RP11, to facilitate degradation of their target mRNAs [36, 37]. In silico analyses of I2ALR support this proposed mechanism; predicted cytoplasmic localization (as well as experimentally validated expression) was consistent with similar IncRNAs (e.g. LINCO1354 [38]) and complementary regions between ITGA2 mRNA and I2ALR were predicted. Interestingly one of these predicted complementary interactions was in the ITGA2 mRNA 5′-UTR while the remaining four were all within the 3′-UTR, regions typically engaged by regulatory RNAs such as miRNAs. The IncRNA RP11 down-regulated SIAH1 and FBXO45 mRNAs by complementary interactions within their CDS and 3′UTR regions [36], and
ITGB2-AS1 downregulated ITGB2 mRNA via an interaction in the mRNAs 5′UTR [39].

The α2 integrin has been targeted by small molecules and antibodies as potential therapies for cancer and other diseases, some reaching phase II trials [40, 41]. There is also interest in lncRNAs as potential novel therapeutics, and the FDA has approved ASO drugs targeting mRNA transcripts to degrade them via RNase H, or as inhibitors of translation [42]. Furthermore, lncRNAs are potential biomarkers of disease, for example prostate cancer antigen 3 in PrCa [43], and HOTAIR as a marker of chemotherapy resistance [44].

To date our limited understanding of mechanisms controlling the complex temporal and context dependent expression of the ITGA2 gene may have hampered our ability to test prospective therapeutics in appropriate contexts. Identification of this lncRNA and its potential role in ITGA2 gene regulation is an important step forward as it offers novel insight into the cell type and context dependent mechanisms controlling α2 integrin expression in tumor development. Whilst the evidence presented suggests that ITGA2 mRNA is the likely direct target of I2ALR, this does not preclude the possibility that other direct targets exist. This could be addressed with experiments examining direct I2ALR interactions. Taken together, these findings shed light on the role of ITGA2 and the nuanced understanding needed for the development and testing of therapeutics targeting this integrin.

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Author contributions  JLD and AFH conceived and designed the work, TJV performed laboratory experiments, analysed the data. TJV prepared the original draft of the work. TJV, JLD and AFH interpreted the data, and participated in revisions and editing of the manuscript.

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Data availability  All data generated or analysed during this study are included in this published article and its supplementary information files. Publicly available data that support the findings of this study are openly available https://www.cbioportal.org/.

Declarations

Conflict of interest  The authors declare they have no conflicts of interest.

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