

RESEARCH ARTICLE

Long non-coding RNA PICSAR decreases adhesion and promotes migration of squamous carcinoma cells by downregulating $\alpha 2\beta 1$ and $\alpha 5\beta 1$ integrin expression

Minna Piipponen^{1,2,3}, Jyrki Heino⁴, Veli-Matti Kähäri^{1,2,3} and Liisa Nissinen^{1,2,3,*}

ABSTRACT

Long non-coding RNAs (lncRNAs) regulate various cellular processes, and they have emerged as potential biomarkers and therapeutic targets in cancer. We have previously characterized the oncogenic role of lncRNA PICSAR (p38 inhibited cutaneous squamous cell carcinoma associated lincRNA) in cutaneous squamous cell carcinoma (cSCC), the most common metastatic skin cancer. In this study, we show that knockdown of PICSAR in cSCC cells upregulates expression of $\alpha 2$, $\alpha 5$ and $\beta 1$ integrins, resulting in increased cell adhesion and decreased cell migration on collagen I and fibronectin. In contrast, overexpression of PICSAR in cSCC cells downregulates expression of $\alpha 2$, $\alpha 5$ and $\beta 1$ integrins on cell surface, resulting in decreased cell adhesion on collagen I and fibronectin and increased cell migration. These results demonstrate a novel mechanism for regulation of the expression of collagen and fibronectin binding integrins by lncRNA PICSAR, leading to altered adhesion and migration of cSCC cells.

This article has an associated First Person interview with the first author of the paper.

KEY WORDS: Long non-coding RNA, PICSAR, Integrin, Adhesion, Cutaneous squamous cell carcinoma, Cancer

INTRODUCTION

Long non-coding RNAs (lncRNAs) are a diverse and widely uncharacterized group of non-coding transcripts involved in cell regulation (Geisler and Collier, 2013). The single-stranded RNA structure makes lncRNAs versatile regulators of gene expression, as they are able to bind to chromatin and other DNA sequences, RNA and proteins (Rinn and Chang, 2012). Recent studies have elucidated the role of various lncRNAs in several malignancies, such as neurological and cardiovascular diseases (Cipolla et al., 2018), but also in cancer progression, suggesting them as important biomarkers and therapeutic targets (Evans et al., 2016; Schmitz et al., 2016). However, the understanding of mechanistic

functions of distinct lncRNAs in growth and invasion of cancer cells is incomplete.

Keratinocyte-derived cutaneous squamous cell carcinoma (cSCC) is the most common metastatic skin cancer, and its incidence is increasing globally (Madan et al., 2010; Ratushny et al., 2012). An important risk factor for cSCC is the exposure to solar ultraviolet light, but the tumor microenvironment plays also a key role in the progression of cSCC (Nissinen et al., 2016). The molecular basis of the progression of cSCC is incompletely understood. We have recently identified and characterized lncRNA PICSAR (p38 inhibited cutaneous squamous cell carcinoma associated lincRNA), which is specifically expressed by tumor cells in cSCC but not by keratinocytes in normal skin *in vivo* (Piipponen et al., 2016). We showed that knockdown of PICSAR inhibits cSCC cell proliferation and migration on an uncoated surface and suppresses growth of human cSCC xenografts *in vivo*, providing evidence for its tumorigenic function (Piipponen et al., 2016).

Here, we show that knockdown of PICSAR in cSCC cells upregulates expression of $\alpha 2$, $\alpha 5$ and $\beta 1$ integrins in cSCC cells, resulting in increased cell adhesion and decreased cell migration both on collagen I and fibronectin. In contrast, overexpression of PICSAR in cSCC cells downregulates expression of $\alpha 2$, $\alpha 5$ and $\beta 1$ integrins on cell surface, resulting in decreased cell adhesion on collagen I and fibronectin and increased cell migration. These results identify a novel mechanistic role for long non-coding RNA PICSAR in modifying the expression of collagen- and fibronectin-binding integrins, and cell adhesion and migration.

RESULTS AND DISCUSSION**Knockdown of PICSAR inhibits cSCC cell migration on collagen I and fibronectin**

We have previously provided the first functional evidence of a tumorigenic lncRNA in cSCC, called PICSAR (p38 inhibited cutaneous squamous cell carcinoma associated lincRNA) (Piipponen et al., 2016). To study the functional role for PICSAR in cell motility, we analyzed real-time cell migration of individual cSCC cells on a collagen I and fibronectin coated surface after PICSAR knockdown. The cell tracking assay showed that silencing of PICSAR decreased migration of cSCC cells on collagen I and fibronectin compared to control siRNA transfected cells (Fig. 1A; Fig. S1A).

Knockdown of PICSAR increases cSCC cell adhesion and formation of lamellipodia

To further examine the role of PICSAR in cell adhesion, cSCC cells were transfected with PICSAR siRNA or control siRNA and plated on collagen I or fibronectin. Real-time cell adhesion was determined using the xCELLigence system. The results showed that PICSAR

¹Department of Dermatology, University of Turku and Turku University Hospital, FI-20520 Turku, Finland. ²Western Cancer Center (FICAN West), University of Turku and Turku University Hospital, FI-20520 Turku, Finland. ³MediCity Research Laboratory, University of Turku, FI-20520 Turku, Finland. ⁴Department of Biochemistry, University of Turku, FI-20500 Turku, Finland.

*Author for correspondence (liisa.nissinen@utu.fi)

 L.N., 0000-0002-6743-6736

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

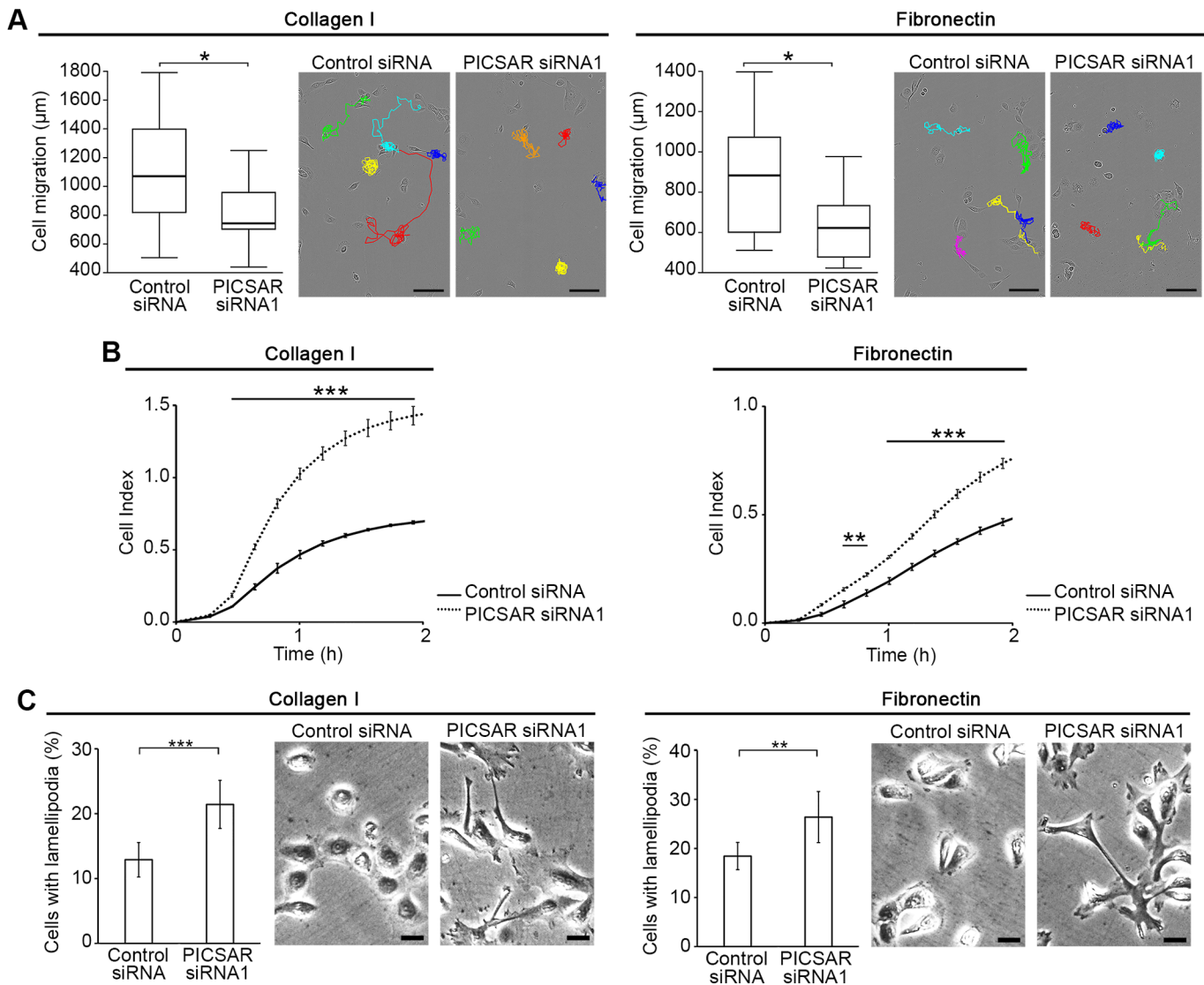


Fig. 1. Knockdown of PICSAR inhibits cSCC cell migration and increases cSCC cell adhesion and formation of lamellipodia. cSCC cells (UT-SCC12A) were transfected with PICSAR siRNA (siRNA1) or control siRNA. (A) Cells were plated on collagen I or fibronectin 72 h after transfection and migration of individual cells was imaged using the IncuCyte ZOOM[®] real-time cell imaging system. Cell tracking ($n=15$) was quantitated using ImageJ software. Median \pm s.d. is shown; $*P<0.05$, Mann–Whitney two-way U -test. Representative images of cell tracking are shown. Scale bars: 100 μm . (B) Cells were plated on collagen I or fibronectin coated 96-well electronic microtiter plate 72 h after transfection and cell adhesion was measured using the xCELLigence system ($n=3$). The cell index indicates the readout of the microelectrode impedance which corresponds to the strength of cell adhesion. Mean \pm s.d. is shown; $**P<0.01$, $***P<0.001$; two-tailed t -test. (C) Cells were plated on collagen I or fibronectin 72 h after transfection and fixed 4 h after plating. The number of lamellipodia containing cells was quantitated from microscopic images with a 20x objective ($n=3$). Representative images of the quantification are shown. Scale bars: 10 μm . Mean \pm s.d. is shown; $**P<0.01$, $***P<0.001$; two-tailed t -test.

knockdown significantly increased adhesion of cSCC cells to collagen I and fibronectin compared to control siRNA transfected cells (Fig. 1B; Figs S1B and S2A). After PICSAR knockdown the morphology of cSCC cells was less spherical than in the control siRNA transfected cultures and cells extended multiple lamellipodia around them (Fig. 1C; Figs S1C and S2B). The relative number of cSCC cells with lamellipodia on collagen I and fibronectin was higher in cultures after PICSAR knockdown, as compared to control siRNA transfected cultures (Fig. 1C; Figs S1C and S2B).

Knockdown of PICSAR increases integrin expression in cSCC cells

To elucidate the mechanistic role of PICSAR in regulating cell adhesion, the differentially expressed genes specifically functioning

in cell adhesion (Winograd-Katz et al., 2014) were further examined from the RNA sequencing of cSCC cells after PICSAR knockdown (Piipponen et al., 2016). We noted that the expression of several of these genes was regulated ($\text{FC log}_2>0.5$) in cSCC cells after PICSAR knockdown, including genes coding for integrins, particularly *ITGA2*, *ITGA5* and *ITGB1*, which code for α_2 , α_5 , and β_1 integrin subunits (Fig. 2A). These integrin subunits form functional heterodimeric $\alpha_2\beta_1$ and $\alpha_5\beta_1$ membrane proteins, which function as cell surface receptors for type I collagen and fibronectin, respectively. These results support our previous bioinformatic analysis of PICSAR knockdown RNA sequencing, which revealed extracellular matrix binding and extracellular matrix-receptor interaction among the top functions associated with differentially expressed genes after PICSAR knockdown (Piipponen et al., 2016).

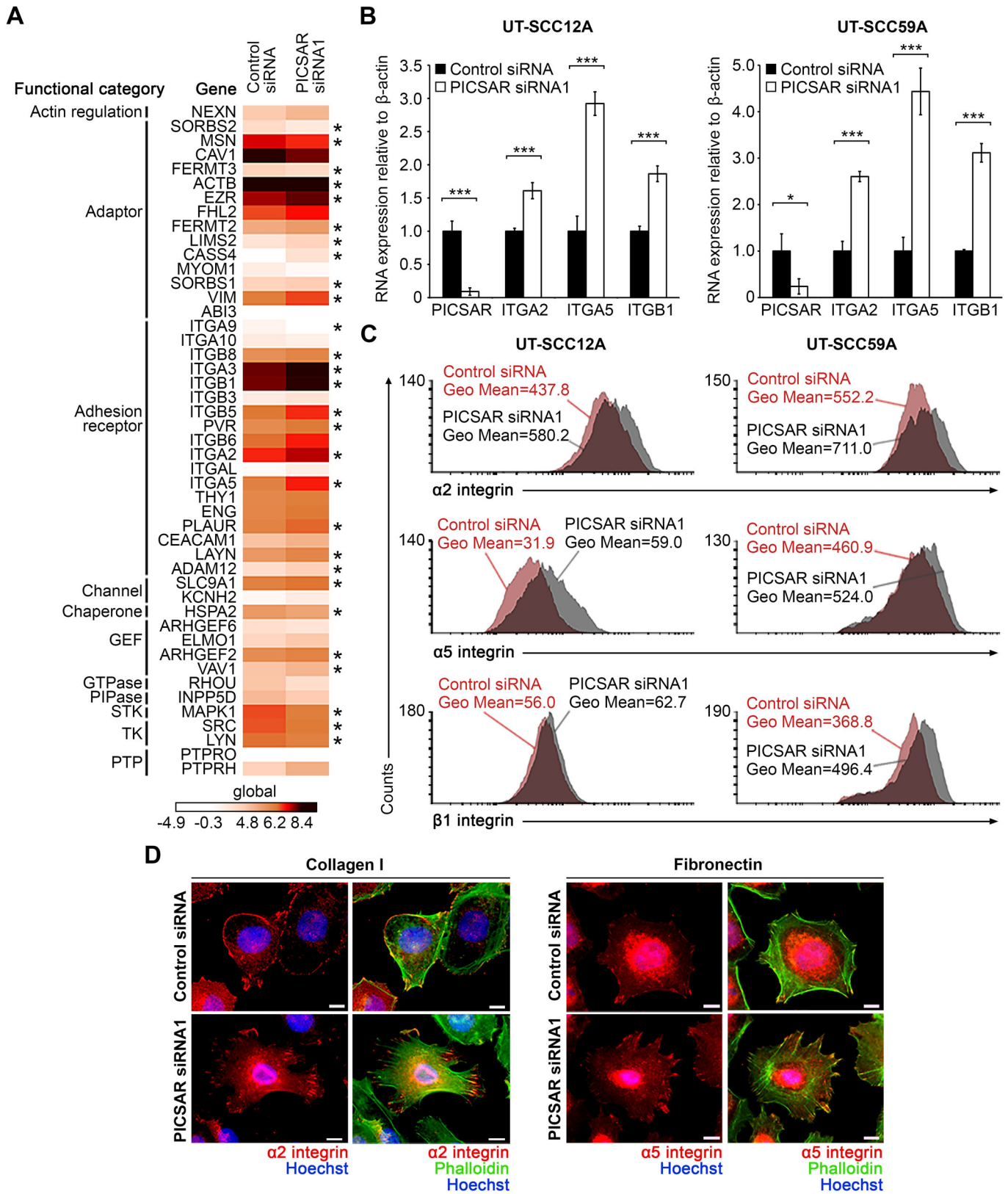


Fig. 2. See next page for legend.

Integrins serve as important regulators of cell migration, differentiation and survival, and they also play a role in cancer progression (Guo and Giancotti, 2004; Heino and Käpylä, 2009). For instance, α 2 β 1 integrin has been shown to exert a tumor

suppressive function in breast and prostate cancer, whereas loss of α 2 β 1 integrin correlates with metastasis in cancer patients and promotes tumor cell intravasation *in vivo* and *in vitro* (Ramirez et al., 2011), indicating that loss of integrin-mediated cell adhesion is an

Fig. 2. Knockdown of PICSAR increases integrin expression in cSCC cells. (A) Three cSCC cell lines (UT-SCC12A, UT-SCC59A, and UT-SCC118) were transfected with PICSAR siRNA (siRNA1) or control siRNA. After 72 h of transfection, whole transcriptome analysis was performed with RNA-seq. Heatmap showing several cell adhesion related genes regulated by PICSAR knockdown ($FC \log_2 > 0.5$). GEF, guanine nucleotide exchange factor; PIPase, phosphatidylinositol phosphatase; STK, serine/threonine specific protein kinase; TK, tyrosine kinase; PTP, protein tyrosine phosphatase; * $P < 0.05$. (B) Levels of PICSAR and $\alpha 2$, $\alpha 5$ and $\beta 1$ integrin mRNAs were determined with qPCR in PICSAR knockdown UT-SCC12A and UT-SCC59A cells ($n=3$). Mean \pm s.d. is shown. * $P < 0.05$, *** $P < 0.001$; two-tailed t -test. (C) Flow cytometry was performed to measure $\alpha 2$, $\alpha 5$ and $\beta 1$ integrin expression on the cell surface in PICSAR knockdown UT-SCC12A and UT-SCC59A cells. (D) UT-SCC12A cells were transfected with PICSAR siRNA or control siRNA and plated on collagen I or fibronectin. Cells were fixed and stained with primary antibodies against $\alpha 2$ or $\alpha 5$ integrins (red), Alexa Fluor[®] 488 conjugated phalloidin (green) and Hoechst (blue). Scale bars: 10 μ m.

important event in invasion and metastasis of cancer cells. Cell migration is a multistep process, which requires focal adhesion disassembly regulated by integrin recycling, and complex coordination of actin cytoskeleton, microtubules and a large group of signaling molecules (Webb et al., 2002; Pellinen and Ivaska, 2006). It is also dependent on the optimal balance in integrin expression, so that increased integrin expression results in increased adhesiveness, as the cells are able to form more bonds to the surrounding extracellular matrix (Palecek et al., 1997).

Quantitation of integrin mRNA levels in cSCC cells after PICSAR knockdown with qPCR showed elevated expression of $\alpha 2$, $\alpha 5$ and $\beta 1$ integrins in cSCC cells after PICSAR knockdown (Fig. 2B; Fig. S3A). Furthermore, flow cytometry analysis showed increased expression of $\alpha 2$ and $\alpha 5$ integrins on the surface of cSCC cells after PICSAR knockdown, compared to the control siRNA transfected cells (Fig. 2C). Expression of $\beta 1$ integrin on the cell surface was increased in UT-SCC59A when using two different PICSAR targeting siRNAs (Fig. 2C; Fig. S3B), whereas in UT-SCC12A cells the effect was less potent after PICSAR knockdown (Fig. 2C). Immunofluorescence staining of $\alpha 2$ and $\alpha 5$ integrins showed similar localization to the cell surface and adhesion sites both in control siRNA and PICSAR siRNA transfected cSCC cells (Fig. 2D).

PICSAR overexpression decreases integrin expression in cSCC cells

To support our findings, cell migration and adhesion was studied in cSCC cells overexpressing PICSAR. First, cSCC cells were stably transfected with PICSAR expression vector and the level of overexpression was verified by qPCR (Fig. 3A). Levels of $\alpha 2$, $\alpha 5$ and $\beta 1$ integrin mRNAs were significantly downregulated in stably PICSAR overexpressing cSCC cells (Fig. 3A). Also, expression of $\alpha 2$, $\alpha 5$ and $\beta 1$ integrins on the cell surface, determined by flow cytometry, was decreased in PICSAR overexpressing cSCC cells (Fig. 3B).

The link between lncRNAs and integrin regulation is not well known and noted only in few studies (Zhang et al., 2014; Kim et al., 2013). Furthermore, the regulation of $\alpha 2\beta 1$ or $\alpha 5\beta 1$ integrins by lncRNAs has not been reported previously. Instead, microRNAs (miRNAs) have been shown to regulate expression of several integrin subunits, for example by targeting the 3'-UTR of integrin mRNAs (Chen et al., 2013). There are reports of lncRNAs targeting and inhibiting specific miRNAs but this competitive endogenous RNA (ceRNA) hypothesis has also been challenged (Thomson and Dinger, 2016). Here, using bioinformatics analysis, we did not find

any potential miRNA-binding sites for PICSAR which could explain the regulation of integrin expression via altered miRNA expression after PICSAR knockdown or PICSAR overexpression in cSCC cells.

Cell adhesion and spreading is decreased by PICSAR overexpression leading to increased migration of cSCC cells

To investigate the effect of PICSAR on cell spreading, PICSAR overexpressing cells were plated on collagen I and fibronectin. PICSAR overexpression resulted in impaired cell spreading and the relative number of spread cells was significantly decreased in PICSAR overexpressing cells compared to control cells (Fig. 3C). Immunofluorescence staining revealed expression of $\alpha 2$ and $\alpha 5$ integrins in PICSAR overexpressing cSCC cells plated on collagen I and fibronectin, but compared to the control cells there were no clear adhesion sites in PICSAR overexpressing cells, likely due to decreased cell spreading (Fig. 3D). In accordance with this, real-time cell adhesion assay revealed decreased adhesion of PICSAR overexpressing cSCC cells on collagen I and fibronectin, as compared to control cells (Fig. 3E). Furthermore, migration of PICSAR overexpressing cSCC cells in wound healing assay was increased compared to control cells (Fig. 3F). These results show for the first time a functional link between a lncRNA, PICSAR, and integrin regulation in cSCC cells leading to changes in the migratory phenotype of these keratinocyte-derived cancer cells.

The role of integrins in growth and differentiation of epidermal keratinocytes is well established, and mutations in integrin genes and changes in integrin expression have been documented in cSCC (Watt, 2002; Janes and Watt, 2006). In the epidermal layer of skin, $\alpha 2$ and $\beta 1$ integrins are abundantly expressed by basal keratinocytes, which are in direct contact with intact basement membrane (Häkkinen et al., 1998), and their expression is downregulated during terminal differentiation of epidermal keratinocytes (Janes and Watt, 2006). Fibronectin receptor $\alpha 5\beta 1$ is not expressed by keratinocytes in normal epidermis, but its expression is upregulated in hyperproliferative keratinocytes, for example in wound healing and in keratinocytes in culture (Larjava et al., 1993).

Activation of proto-oncogene Src has been linked to malignant transformation of tumor cells and in cSCC progression (Alt-Holland et al., 2011). Src also functions in other cellular processes such as cell adhesion, and it is an important regulator of integrin-mediated cell spreading and adhesion via direct interaction with integrin cytoplasmic domains transducing signals downstream of these cell surface receptors (Playford and Schaller, 2004). Moreover, Src plays an important role in cell motility by regulating focal adhesion turnover (Fincham and Frame, 1998). Introduction of viral Src (v-Src), a constitutively active variant of cellular Src (c-Src), into normal cells leads to disruption of cell-matrix adhesions and to cells adopting a rounded phenotype (Wang and Goldberg, 1976; Fincham et al., 1995). Both v-Src and c-Src are translocated by actin-dependent manner to focal adhesions where they induce activation of focal adhesion kinase (FAK) and disruption of cellular focal adhesions (Timpson et al., 2001; Fincham and Frame, 1998). In contrast, catalytically inactive c-Src-mutant suppresses FAK activation followed by increased actin stability and formation of enlarged focal adhesions, which eventually leads to decreased cell motility (Fincham and Frame, 1998). Here, decreased expression of Src in cSCC cells was noted after PICSAR knockdown (Fig. 3G; Fig. S4A,B). Moreover, expression of Src was markedly increased after PICSAR overexpression in cSCC cells (Fig. 3G; Fig. S4C), providing further evidence for a regulatory link between PICSAR

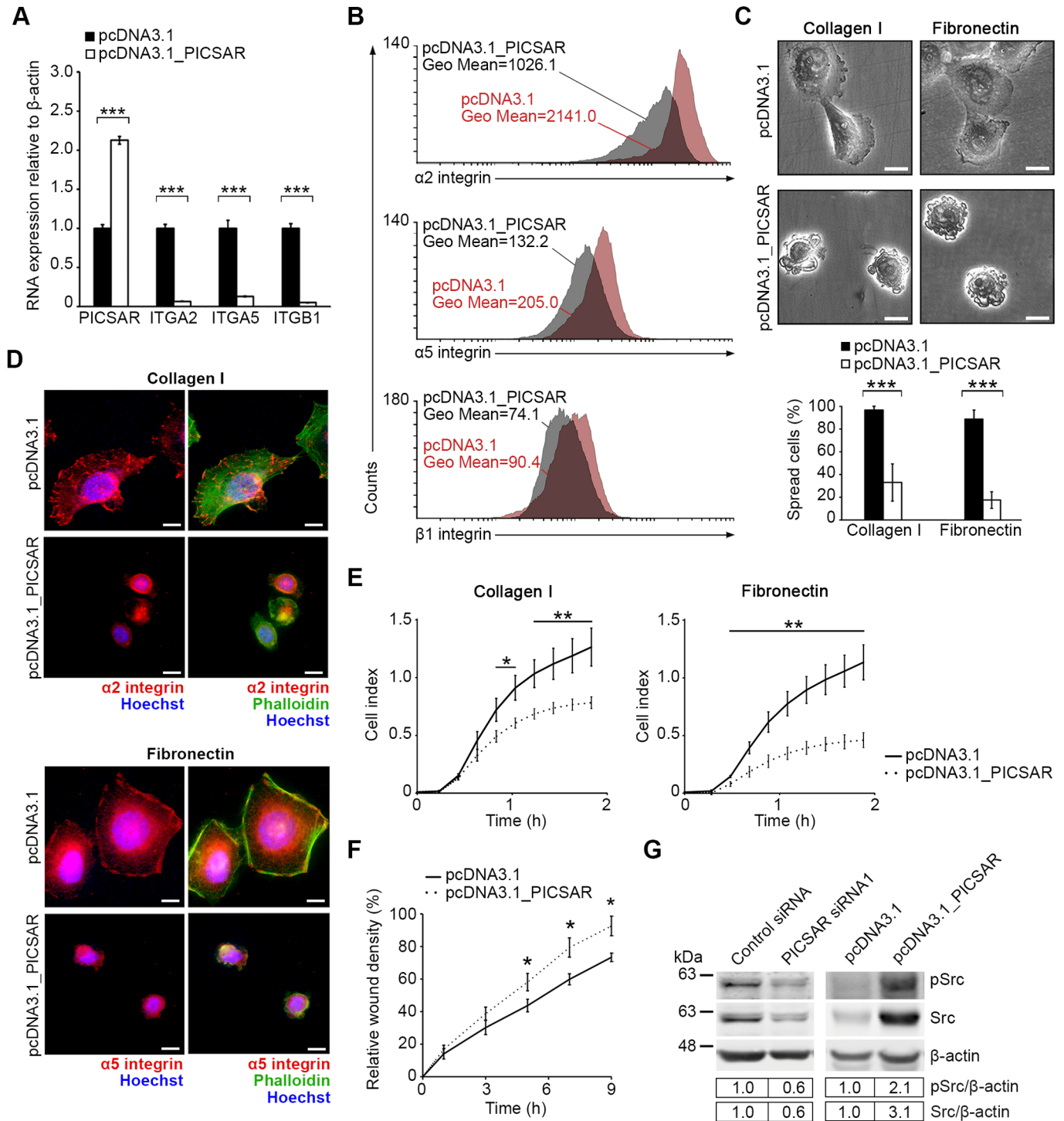


Fig. 3. PICSAR overexpression decreases cell adhesion and spreading, and increases migration of cSCC cells by regulating integrin expression. UT-SCC59A cells were transfected with PICSAR expression construct (pcDNA3.1_PICSAR) or empty vector (pcDNA3.1) and selective pressure of cell pools was maintained by Geneticin. (A) Expression of PICSAR and $\alpha 2$, $\alpha 5$ and $\beta 1$ integrin mRNAs was measured using qPCR ($n=3$). Mean \pm s.d. is shown. (B) Flow cytometry was used to measure $\alpha 2$, $\alpha 5$ and $\beta 1$ integrin expression on the cell surface. (C) PICSAR overexpressing and control UT-SCC59A cells were plated on collagen I and fibronectin and the number of spread cells was quantitated from microscopic images with a 20x objective ($n=3$). Scale bars: 10 μ m. Mean \pm s.d. is shown. Representative images of the quantification are shown. (D) PICSAR overexpressing and control UT-SCC59A cells were plated on collagen I or fibronectin and stained with primary antibodies against $\alpha 2$ or $\alpha 5$ integrin (red), Alexa Fluor[®] 488 conjugated phalloidin (green) and Hoechst (blue). Scale bars: 10 μ m. (E) Adhesion of PICSAR overexpressing and control UT-SCC59A cells on collagen I and fibronectin was measured using the xCELLigence system ($n=3$). Mean \pm s.d. is shown. (F) PICSAR overexpressing and control UT-SCC59A cells were plated on a 96-well plate and incubated for 6 h with 1 mM hydroxyurea to prevent cell proliferation. Cell monolayer was scratched and cells were imaged using the IncuCyte ZOOM[®] real-time cell imaging system ($n=5$). Mean \pm s.e.m. is shown. (G) Protein levels for phosphorylated and total Src were determined by western blotting analysis of PICSAR siRNA (siRNA1) transfected and stably PICSAR overexpressing UT-SCC59A cells. The representative image and quantification shown are from one individual experiment. β -Actin was used as a reference. * $P<0.05$, ** $P<0.01$, *** $P<0.001$; two-tailed t -test.

and Src. These observations are consistent with previous studies, where Src has been shown to have an important role in cell adhesion and motility by regulating focal adhesion assembly and turnover (Fincham and Frame, 1998; Fincham et al., 1995; Wang and Goldberg, 1976; Timpson et al., 2001). Furthermore, the rate of cell migration correlates with the levels of integrin and ligand expression and changes in the affinity of integrin–ligand binding (Palecek et al., 1997). Cell adhesion increases when integrin expression increases, which contributes to a higher number of integrin–ligand bonds and a decreased migration rate (Palecek et al., 1997). Relatively small changes in integrin expression can lead to substantial changes in cell migration speed (Palecek et al., 1997). In addition, Src family kinases have been shown to regulate cell–matrix adhesion by modulating integrin affinity (Li et al., 2002). It is conceivable that optimal cell migration is dependent on the balance of integrin expression and Src activity for functional cell–matrix adhesion dynamics.

Progression of cSCCs is characterized by an alteration of basement membrane structure at an early stage, and by an eventual loss of basement membrane at invasive stage (Karpinen et al., 2016). There is also evidence that the cellular expression of lncRNAs is dependent on the composition and structure of the extracellular matrix (Zhuang et al., 2013; Li et al., 2017), indicating that the lncRNA expression varies depending on the activation of different adhesion receptors. We have previously noted that PICSAR is specifically expressed by tumor cells in cSCC, but not by keratinocytes in normal skin *in vivo* (Piipponen et al., 2016). It is therefore possible that during malignant transformation of epidermal keratinocytes, induction of PICSAR expression negatively regulates integrin expression, allowing detachment of cSCC cells from the basement membrane and invasion through an underlying dermal layer rich in collagen I.

The results of the present study show that PICSAR knockdown results in increased expression of $\alpha 2\beta 1$ and $\alpha 5\beta 1$ integrins on the cell surface, which explains the decreased migration of cSCC cells after PICSAR knockdown when cells adhere more efficiently on a collagen I and fibronectin coated surface. This hypothesis is further supported by experiments with PICSAR overexpressing cSCC cells, where we noted a decrease in integrin expression, resulting in decreased cell adhesion on collagen I and fibronectin, and increased cell migration. These results indicate a new mechanism for PICSAR in invasive cSCC by regulating cell migration by modifying the expression of collagen and fibronectin binding integrins.

MATERIALS AND METHODS

Cell cultures

Cutaneous SCC cell lines (UT-SCC12A and UT-SCC59) were established from surgically removed primary SCCs of the skin in Turku University Hospital (Riihilä et al., 2015) and cultured as previously described (Riihilä et al., 2015; Farshchian et al., 2015). The use of tumor samples was approved by the Ethics Committee of the Hospital District of Southwest Finland. All participants gave their written informed consent and the study was carried out with the permission of Turku University Hospital, according to the Declaration of Helsinki. The authenticity of all cSCC cell lines has been verified by short tandem repeat profiling (Farshchian et al., 2016).

siRNA gene knockdown

cSCC cells were cultured to 50% confluence and transfected with negative control siRNA (AllStars Negative Control siRNA, Qiagen) and following commercially available siRNAs (75 nM, Qiagen) targeting two distinct areas in PICSAR 3' end, as previously described (Piipponen et al., 2016): Hs_C21orf113_1, 5'-CACGGCCAACGTGGAGCTCTA-3' (siRNA1); Hs_C21orf113_3, 5'-CTGCAGTCACTTCACAGTGAA-3' (siRNA2).

Recombinant PICSAR expression in cSCC cells

Specific primers for PICSAR variant expressed in cSCC cells were designed using Primer-BLAST (Ye et al., 2012) and purchased from Oligomer (Metabion International AG, Steinkirchen, Germany): forward primer 5'-CTGGCTCACCTGGCACTG-3', reverse primer 5'-CACCTAAGCAATGCAGAGAGG-3'. This fragment was amplified by PCR with Q5[®] High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, USA) using cSCC cell derived cDNA as template. PCR reaction was cycled at 98°C for 30 s and 40 cycles of amplification (98°C 10 s, 66°C 30 s and 72°C 30 s) followed by extension at 72°C for 10 min. The amplified DNA fragment was cloned into pcDNA3.1(-) (Invitrogen) containing the neomycin resistance gene. The construct was further sequenced to verify the orientation and integrity of the ligated PICSAR insert. cSCC cells were transfected with PICSAR expression construct (pcDNA3.1_PICSAR) or empty vector (pcDNA3.1) using Lipofectamine 3000 transfection reagent (Invitrogen) and transfected cells were selected with 1 µg/ml Geneticin (G418 sulfate, Invitrogen). Selective pressure of PICSAR overexpressing and control cell pools was maintained by using 500 µg/ml Geneticin.

Cell migration assays

An ImageLock[™] 96-well plate (Essen BioScience, Ann Arbor, USA) was coated with fibronectin (Merck Millipore, Darmstadt, Germany) or collagen I (Advanced BioMatrix, San Diego, USA) (both 5 µg/cm²). PICSAR siRNA and control siRNA treated cSCC cells were plated 72 h after siRNA transfection and cells were imaged using the IncuCyte ZOOM[®] real-time cell imaging system (Essen BioScience). Individual migrating cells ($n=15$) were tracked ($t=20$ h) using the ImageJ software (Schneider et al., 2012). To examine cell migration in a wound healing assay, PICSAR expression construct (pcDNA3.1_PICSAR) and control expression vector (pcDNA3.1) transfected cSCC cells were plated on an ImageLock[™] 96-well plate and incubated for 6 h with 1 mM hydroxyurea (Sigma-Aldrich) to prevent cell proliferation. Cell monolayer was scratched with IncuCyte wound-maker (Essen BioScience) simultaneously in all wells and incubation was continued in 1% fetal calf serum (FCS) containing growth media with 0.5 mM hydroxyurea. Cells were imaged every 10 min using the IncuCyte ZOOM[®] and the results were analyzed with the IncuCyte ZOOM 2014A software (Essen BioScience).

Cell adhesion and spreading assay

To study dynamic cell adhesion PICSAR siRNA and control siRNA treated cSCC cells (72 h after transfection), or PICSAR expression construct (pcDNA3.1_PICSAR) and control expression vector (pcDNA3.1) transfected cSCC cells were seeded on a collagen I or fibronectin coated (5 µg/cm²) E-Plate 96 (Roche Applied Sciences) and cell adhesion was measured using the xCELLigence real-time cell analyzer (RTCA; Roche Applied Sciences). The cell index indicates the readout of the microelectrode impedance, which corresponds to the strength of cell adhesion. The formation of lamellipodia was studied by microscopic quantitation in PICSAR siRNA and control siRNA treated cSCC cells 72 h after transfection. Cell spreading was studied in stably PICSAR overexpressing cSCC cells by microscopic quantitation. Cells were plated on collagen I or fibronectin coated 96-wells, and fixed 4 h after plating [8% formaldehyde, 10% sucrose in phosphate buffer saline (PBS) supplemented with 1 mM MgSO₄ and 1 mM CaCl₂]. The number of cells with lamellipodia or the number of spread cells was compared to the total cell number in each well ($n=3$) with a 20x objective.

Visualization of RNA sequencing and quantitative real-time PCR

Morpheus software was used for gene expression visualization and to generate heatmaps (<https://software.broadinstitute.org/morpheus>) from RNA-seq data previously published by us (GEO accession number GSE77950) (Piipponen et al., 2016). Primers and probes for PICSAR and $\alpha 2$, $\alpha 5$ and $\beta 1$ integrins were designed as previously described (Piipponen et al., 2016; Mirtti et al., 2006). The sequences for primers and probes are listed in the supplementary material online (Table S1). All qPCR reactions were performed using the QuantStudio 12K Flex (Thermo Fisher Scientific) system at the Finnish Functional Genomics Centre (FFGC) in Turku, Finland. Samples were analyzed using the standard curve method in

three parallel reactions with threshold cycle (Ct) values <5% of the mean Ct. β -Actin mRNA was used as reference.

Flow cytometry

PICSAR siRNA and control siRNA treated cSCC cells (72 h after transfection), or PICSAR expression construct (pcDNA3.1_PICSAR) and control expression vector (pcDNA3.1) transfected cSCC cells were analyzed for the levels of cell surface integrins $\alpha 2$, $\alpha 5$ and $\beta 1$. Cells were detached from plates by using 0.25% Trypsin-Ethylenediaminetetraacetic acid (EDTA) and incubating in +37° for 5–10 min. Detached cells, 700,000 cells per sample, were suspended in blocking buffer in 1% FCS-PBS and incubated on ice for 30 min. No fixation or permeabilization was done to the cells to prevent intracellular antigen staining. After blocking, cells were stained with monoclonal antibodies (all in 1:100 dilution) specific for integrin $\alpha 2$ (555668), integrin $\alpha 5$ (555615) and integrin $\beta 1$ (553715) (all from BD Biosciences) in 1% FCS-PBS for 1 h in +4°C. Cells were washed with PBS and highly precross-absorbed Alexa Fluor® 488 conjugated goat anti-mouse antibody (Invitrogen) was used as secondary antibody in 1:200 dilution and cells were analyzed using FACSCalibur flow cytometer (BD Biosciences). The results were analyzed with Flowing Software 2.5.1 (Turku Centre for Biotechnology, University of Turku, Finland, www.flowingsoftware.com).

Immunofluorescence assays

PICSAR siRNA and control siRNA treated cSCC cells (72 h after transfection), or PICSAR expression construct (pcDNA3.1_PICSAR) and control expression vector (pcDNA3.1) transfected cSCC cells were plated on cell culture plates coated with collagen I or fibronectin. Cells were fixed 4 h after plating with 4% paraformaldehyde-PBS, blocked with 3% bovine serum albumin (BSA) containing PBS and permeabilized with 0.2% Triton X-100-PBS. Cells were labeled with primary antibodies against integrin $\alpha 2$ (MCA2025, Serotec, Raleigh, USA) or integrin $\alpha 5$ (AB1949, Merck Millipore) in 1:100 dilution in blocking solution. Alexa Fluor® 568 conjugated goat anti-mouse or anti-rabbit antibody (Invitrogen) was used as secondary antibody in 1:200 dilution together with Alexa Fluor® 488 conjugated phalloidin in 1:50 dilution (Invitrogen). Hoechst (Invitrogen) was used to visualize nuclei. Cells were mounted on culture plates with Mowiol-DABCO (Sigma-Aldrich) and samples were examined with Zeiss AxioVert 200 M (Carl Zeiss, Jena, Germany) fluorescence microscope.

Western blot analysis

Cell lysates of PICSAR and control siRNA transfected cultures, and PICSAR overexpressing and pcDNA3.1 vector containing cSCC cells were analyzed with antibodies specific for phosphorylated Src (pSrc, 2101) and total Src (2108, both from Cell Signaling Technology) in 1:1000 dilution. β -Actin (A1978, Sigma-Aldrich) expression level was used as loading control. Protein expression was quantitated using fluorescently labeled secondary antibodies in 1:15000 dilution (LI-COR Biosciences, Lincoln, NE) and the LI-COR Odyssey® CLx fluorescent imaging system.

Bioinformatic analysis of miRNA binding sites

To analyze potential miRNA target sites in PICSAR, following computational programs with different algorithms for miRNA-binding prediction were used; miRWalk (<http://mirwalk.umm.uni-heidelberg.de/>) (Dweep and Gretz, 2015), TargetScan (<http://www.targetscan.org/>) (Agarwal et al., 2015) and DIANA-IncBase v2 (www.microrna.gr/LncBase) (Paraskevopoulou et al., 2015).

Statistical analysis

Statistical analysis was performed using SPSS Statistics for Windows, v. 20.0. (IBM, Armonk, USA). Unpaired two-tailed *t*-test and Mann–Whitney two-way *U*-test were used. Statistical analysis for RNA-seq analysis was performed using the Limma package (Pipponen et al., 2016).

Acknowledgements

We thank Johanna Markola for expert technical assistance, Dr Reidar Grénman for cSCC cell lines and tumor samples, and the Cell Imaging Core of the Turku Centre for Biotechnology for their expertise and service. We thank the Bioinformatics Unit of

Turku Centre for Biotechnology for the RNA-seq data analysis. The Unit is supported by University of Turku, Åbo Akademi University and Biocenter Finland.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.P., V.-M.K., L.N.; Formal analysis: M.P.; Investigation: M.P.; Resources: J.H., V.-M.K.; Data curation: M.P.; Writing - original draft: M.P., J.H., V.-M.K., L.N.; Writing - review & editing: M.P., J.H., V.-M.K., L.N.; Visualization: M.P.; Supervision: V.-M.K., L.N.; Project administration: V.-M.K.; Funding acquisition: V.-M.K., L.N.

Funding

This study was supported by the Finnish Cancer Research Foundation, Sigrid Jusélius Foundation, Jane ja Aatos Erkko Foundation, Turku University Hospital State Research Funding (project 13336), Turku Doctoral Programme of Molecular Medicine, the Kymenlaakso Regional Fund of the Finnish Cultural Foundation and Turku University Foundation.

Data availability

RNA seq data is available online at the Gene Expression Omnibus (GEO accession number GSE77950).

Supplementary information

Supplementary information available online at <http://bio.biologists.org/lookup/doi/10.1242/bio.037044.supplemental>

References

- Agarwal, V., Bell, G. W., Nam, J.-W. and Bartel, D. P. (2015). Predicting effective microRNA target sites in mammalian mRNAs. *Elife* **4**, E05005.
- Alt-Holland, A., Sowalsky, A. G., Szwec-Levin, Y., Shamis, Y., Hatch, H., Feig, L. A. and Garlick, J. A. (2011). Suppression of E-cadherin function drives the early stages of Ras-induced squamous cell carcinoma through upregulation of FAK and Src. *J. Invest. Dermatol.* **131**, 2306–2315.
- Chen, W., Harbeck, M. C., Zhang, W. and Jacobson, J. R. (2013). MicroRNA regulation of integrins. *Transl. Res.* **162**, 133–143.
- Cipolla, G. A., de Oliveira, J. C., Salviano-Silva, A., Lobo-Alves, S. C., Lemos, D. S., Oliveira, L. C., Jucoski, T. S., Mathias, C., Pedrosa, G. A., Zambalde, E. P. et al. (2018). Long non-coding RNAs in multifactorial diseases: another layer of complexity. *Noncoding RNA* **4**, E13.
- Dweep, H. and Gretz, N. (2015). miRWalk2.0: a comprehensive atlas of microRNA-target interactions. *Nat. Methods* **12**, 697.
- Evans, J. R., Feng, F. Y. and Chinnaiyan, A. M. (2016). The bright side of dark matter: lncRNAs in cancer. *J. Clin. Invest.* **126**, 2775–2782.
- Farshchian, M., Nissinen, L., Siljamäki, E., Riihilä, P., Toriseva, M., Kivisaari, A., Ala-Aho, R., Kallajoki, M., Veräjänkorva, E., Honkanen, H.-K. et al. (2015). EphB2 promotes progression of cutaneous squamous cell carcinoma. *J. Invest. Dermatol.* **135**, 1882–1892.
- Farshchian, M., Nissinen, L., Grénman, R. and Kähäri, V.-M. (2016). Dasatinib promotes apoptosis of cutaneous squamous carcinoma cells by regulating activation of ERK1/2. *Exp. Dermatol.* **26**, 89–92.
- Fincham, V. J. and Frame, M. C. (1998). The catalytic activity of Src is dispensable for translocation to focal adhesions but controls the turnover of these structures during cell motility. *EMBO J.* **17**, 81–92.
- Fincham, V. J., Wyke, J. A. and Frame, M. C. (1995). v-Src-induced degradation of focal adhesion kinase during morphological transformation of chicken embryo fibroblasts. *Oncogene* **10**, 2247–2252.
- Geisler, S. and Coller, J. (2013). RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. *Nat. Rev. Mol. Cell Biol.* **14**, 699–712.
- Guo, W. and Giancotti, F. G. (2004). Integrin signalling during tumour progression. *Nat. Rev. Mol. Cell Biol.* **5**, 816–826.
- Häkkinen, L., Westermarck, J., Johansson, N., Aho, H., Peltonen, J., Heino, J. and Kähäri, V. M. (1998). Suprabasal expression of epidermal $\alpha 2\beta 1$ and $\alpha 3\beta 1$ integrins in skin treated with topical retinoic acid. *Br. J. Dermatol.* **138**, 29–36.
- Heino, J. and Käpylä, J. (2009). Cellular receptors of extracellular matrix molecules. *Curr. Pharm. Des.* **15**, 1309–1317.
- Janes, S. M. and Watt, F. M. (2006). New roles for integrins in squamous-cell carcinoma. *Nat. Rev. Cancer* **6**, 175–183.
- Karppinen, S.-M., Honkanen, H.-K., Heljasvaara, R., Riihilä, P., Autio-Harmainen, H., Sormunen, R., Harjunen, V., Väisänen, M.-R., Väisänen, T., Hurskainen, T. et al. (2016). Collagens XV and XVIII show different expression and localisation in cutaneous squamous cell carcinoma: type XV appears in tumor stroma, while XVIII becomes upregulated in tumor cells and lost from microvessels. *Exp. Dermatol.* **25**, 348–354.

- Kim, D., Song, J., Han, J., Kim, Y., Chun, C.-H. and Jin, E.-J. (2013). Two non-coding RNAs, MicroRNA-101 and HOTTIP contribute cartilage integrity by epigenetic and homeotic regulation of integrin- $\alpha 1$. *Cell. Signal.* **25**, 2878-2887.
- Larjava, H., Salo, T., Haapasalmi, K., Kramer, R. H. and Heino, J. (1993). Expression of integrins and basement membrane components by wound keratinocytes. *J. Clin. Invest.* **92**, 1425-1435.
- Li, L., Okura, M. and Imamoto, A. (2002). Focal adhesions require catalytic activity of Src family kinases to mediate integrin-matrix adhesion. *Mol. Cell. Biol.* **22**, 1203-1217.
- Li, M., Li, X., Zhuang, Y., Flemington, E. K., Lin, Z. and Shan, B. (2017). Induction of a novel isoform of the lncRNA HOTAIR in Claudin-low breast cancer cells attached to extracellular matrix. *Mol. Oncol.* **11**, 1698-1710.
- Madan, V., Lear, J. T. and Szeimies, R.-M. (2010). Non-melanoma skin cancer. *Lancet* **375**, 673-685.
- Mirtti, T., Nylund, C., Lehtonen, J., Hiekkanen, H., Nissinen, L., Kallajoki, M., Alanen, K., Gullberg, D. and Heino, J. (2006). Regulation of prostate cell collagen receptors by malignant transformation. *Int. J. Cancer* **118**, 889-898.
- Nissinen, L., Farshchian, M., Riihilä, P. and Kähäri, V.-M. (2016). New perspectives on role of tumor microenvironment in progression of cutaneous squamous cell carcinoma. *Cell Tissue Res.* **365**, 691-702.
- Palecek, S. P., Loftus, J. C., Ginsberg, M. H., Lauffenburger, D. A. and Horwitz, A. F. (1997). Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. *Nature* **385**, 537-540.
- Paraskevopoulou, M. D., Vlachos, I. S., Karagkouni, D., Georgakilas, G., Kanellos, I., Vergoulis, T., Zagganas, K., Tsanakas, P., Floros, E., Dalamagas, T. et al. (2015). DIANA-LncBase v2: indexing microRNA targets on non-coding transcripts. *Nucleic Acids Res.* **44**, D231-D238.
- Pellinen, T. and Ivaska, J. (2006). Integrin traffic. *J. Cell Sci.* **119**, 3723-3731.
- Piipponen, M., Nissinen, L., Farshchian, M., Riihilä, P., Kivisaari, A., Kallajoki, M., Peltonen, J., Peltonen, S. and Kähäri, V.-M. (2016). Long noncoding RNA PICSAR promotes growth of cutaneous squamous cell carcinoma by regulating ERK1/2 activity. *J. Invest. Dermatol.* **136**, 1541-1542.
- Playford, M. P. and Schaller, M. D. (2004). The interplay between Src and integrins in normal and tumor biology. *Oncogene* **23**, 7928-7946.
- Ramirez, N. E., Zhang, Z., Madamanchi, A., Boyd, K. L., O'Rear, L. D., Nashabi, A., Li, Z., Dupont, W. D., Zijlstra, A. and Zutter, M. M. (2011). The $\alpha 2\beta 1$ integrin is a metastasis suppressor in mouse models and human cancer. *J. Clin. Invest.* **121**, 226-237.
- Ratushny, V., Gober, M. D., Hick, R., Ridky, T. W. and Seykora, J. T. (2012). From keratinocyte to cancer: the pathogenesis and modeling of cutaneous squamous cell carcinoma. *J. Clin. Invest.* **122**, 464-472.
- Riihilä, P., Nissinen, L., Farshchian, M., Kivisaari, A., Ala-aho, R., Kallajoki, M., Grénman, R., Meri, S., Peltonen, S., Peltonen, J. et al. (2015). Complement factor I promotes progression of cutaneous squamous cell carcinoma. *J. Invest. Dermatol.* **135**, 579-588.
- Rinn, J. L. and Chang, H. Y. (2012). Genome regulation by long noncoding RNAs. *Annu. Rev. Biochem.* **81**, 145-166.
- Schmitz, S. U., Grote, P. and Herrmann, B. G. (2016). Mechanisms of long noncoding RNA function in development and disease. *Cell. Mol. Life Sci.* **73**, 2491-2509.
- Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671-675.
- Thomson, D. W. and Dinger, M. E. (2016). Endogenous microRNA sponges: evidence and controversy. *Nat. Rev. Genet.* **17**, 272-283.
- Timpson, P., Jones, G. E., Frame, M. C. and Brunton, V. G. (2001). Coordination of cell polarization and migration by the Rho family GTPases requires Src tyrosine kinase activity. *Curr. Biol.* **11**, 1836-1846.
- Wang, E. and Goldberg, A. R. (1976). Changes in microfilament organization and surface topography upon transformation of chick embryo fibroblasts with Rous sarcoma virus. *Proc. Natl. Acad. Sci. USA* **73**, 4065-4069.
- Watt, F. M. (2002). Role of integrins in regulating epidermal adhesion, growth and differentiation. *EMBO J.* **21**, 3919-3926.
- Webb, D. J., Parsons, J. T. and Horwitz, A. F. (2002). Adhesion assembly, disassembly and turnover in migrating cells—over and over and over again. *Nat. Cell Biol.* **4**, E97-E100.
- Winograd-Katz, S. E., Fässler, R., Geiger, B. and Legate, K. R. (2014). The integrin adhesome: from genes and proteins to human disease. *Nat. Rev. Mol. Cell Biol.* **4**, 273-288.
- Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S. and Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics* **13**, 134.
- Zhang, X., Weissman, S. M. and Newburger, P. E. (2014). Long intergenic non-coding RNA HOTAIRM1 regulates cell cycle progression during myeloid maturation in NB4 human promyelocytic leukemia cells. *RNA Biol.* **11**, 777-787.
- Zhuang, Y., Wang, X., Nguyen, H. T., Zhuo, Y., Cui, X., Fewell, C., Flemington, E. K. and Shan, B. (2013). Induction of long intergenic non-coding RNA HOTAIR in lung cancer cells by type I collagen. *J. Hematol. Oncol.* **6**, 35.

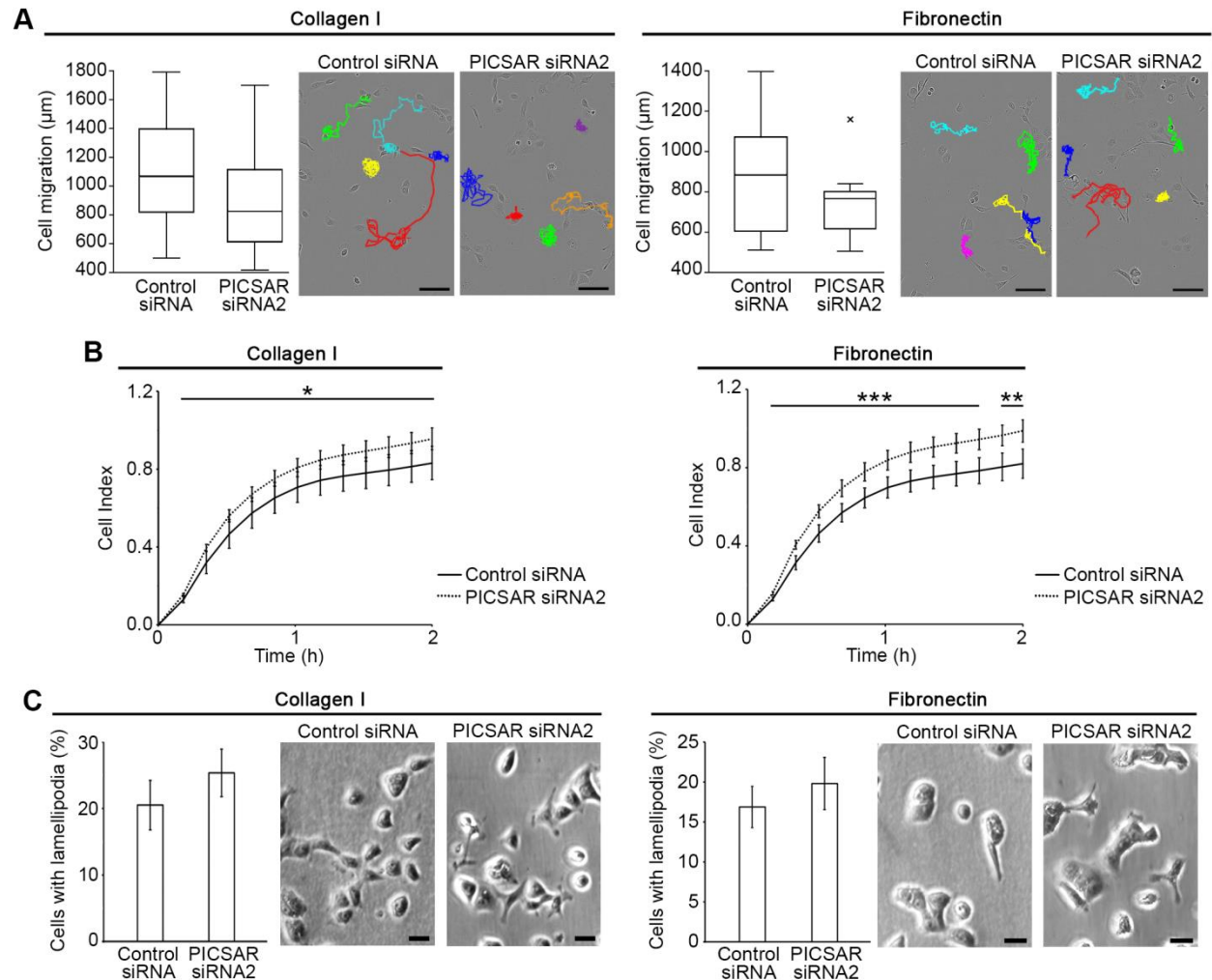


Fig. S1. Knockdown of PICSAR inhibits cSCC cell migration and increases cSCC cell adhesion and formation of lamellipodia. cSCC cells (UT-SCC12A) were transfected with PICSAR siRNA (siRNA2) or control siRNA. (A) cSCC cells were plated on collagen I or fibronectin 72 hours after transfection and migration of individual cells was imaged using the IncuCyte ZOOM[®] real-time cell imaging system. Cell tracking (n=15) was quantitated using ImageJ software. Median \pm s.d. is shown. Representative images of cell tracking are shown. Scale bar=100 μm . (B) cSCC cells were plated on collagen I or fibronectin coated 96-well electronic microtiter plate 72 hours after transfection and cell adhesion was measured using the xCELLigence system (n=3). Mean \pm s.d. is shown; * P <0.05, ** P <0.01, *** P <0.001; two-tailed t -test. (C) cSCC were plated on collagen I or fibronectin 72 hours after transfection and fixed 4 hours after plating. The number of lamellipodia containing cells was quantitated from microscopic images at 20x magnification (n=3). Representative images of the quantitation are shown. Scale bar=10 μm . Mean \pm s.d. is shown.

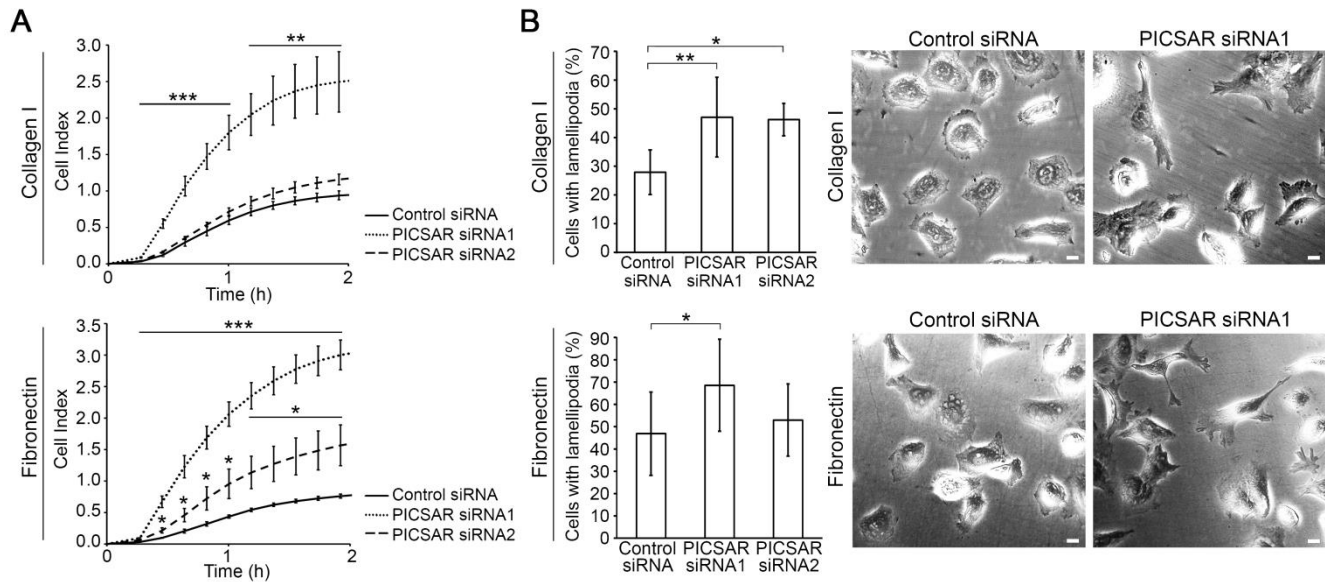


Fig. S2. Knockdown of PICSAR increases cSCC cell adhesion and formation of lamellipodia.

cSCC cells (UT-SCC59A) were transfected with PICSAR siRNAs (siRNA1 or siRNA2) or control siRNA. (A) Cells were plated on collagen I or fibronectin coated 96-well electronic microtiter plate 72 hours after transfection and cell adhesion was measured using the xCELLigence system (n=3). Mean±s.d. is shown. (B) After 72 hours of transfection cells were plated on collagen I or fibronectin coated wells. Cells were fixed 4 hours after plating and the number of lamellipodia containing cells was quantitated from microscopic images (n=3) at 20x magnification (left panel). Mean±s.d. is shown. Representative images of the quantification are shown (right panel). Scale bar=10 μm. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; two-tailed t -test.

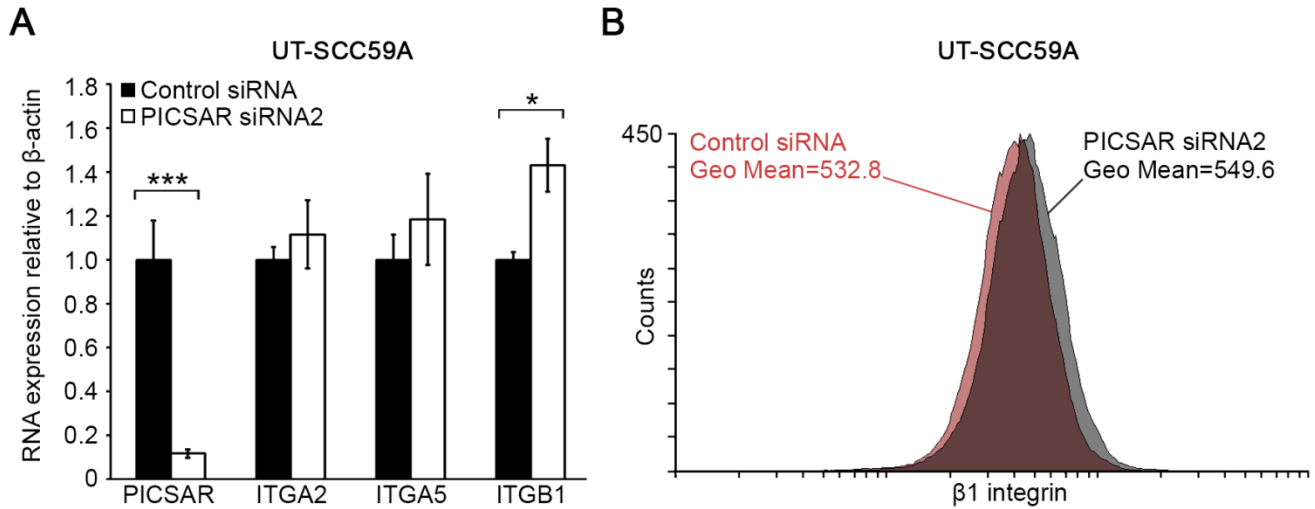


Fig. S3. Knockdown of PICSAR increases integrin expression in cSCC cells. cSCC cells (UT-SCC59A) were transfected with PICSAR siRNA (siRNA2) or control siRNA and incubated for 72 hours. (A) Levels of PICSAR and α 2, α 5 and β 1 integrin mRNAs were determined with qPCR in PICSAR knockdown cells (n=3). Mean \pm s.d. is shown. * P <0.05, *** P <0.001; two-tailed t -test. (B) Flow cytometry was performed to measure β 1 integrin expression on the cell surface in PICSAR knockdown cells.

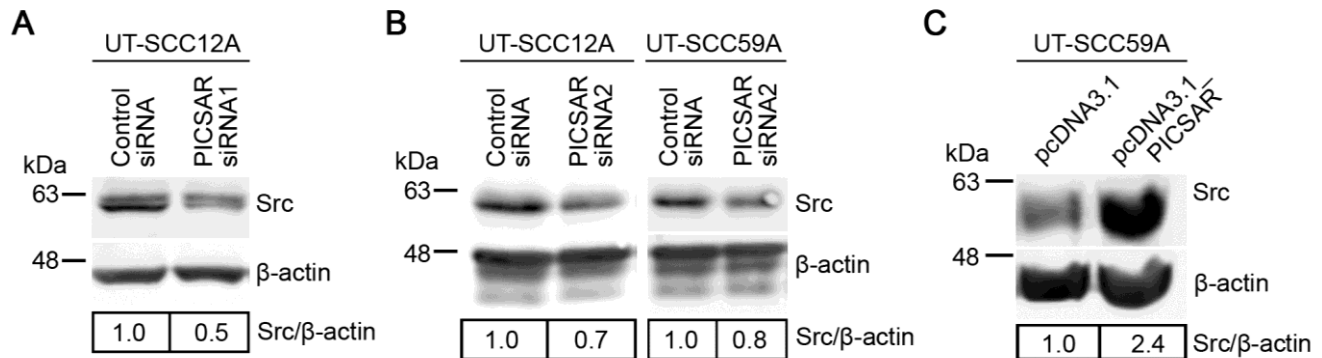


Fig. S4. Expression of Src is regulated by PICSAR in cSCC cells. (A) cSCC cells (UT-SCC12A) were transfected with PICSAR siRNA (siRNA1) or control siRNA. After 72 hours of transfection protein level for Src was determined by Western blotting analysis. (B) cSCC cells (UT-SCC12A and UT-SCC59A) were transfected with PICSAR siRNA (siRNA2) or control siRNA and protein level for Src was determined by Western blotting analysis 72 hours after transfection. (C) Protein level for Src was determined by Western blotting analysis of stably PICSAR overexpressing UT-SCC59A cells. The representative image and quantification shown are from one individual experiment. β -Actin was used as loading control.

Table S1. List of specific primers and probes for qPCR.

Gene		Sequence
<i>PICSAR</i>	Forward	5'-TGCCTGGACTTTCAAGAGGTAA-3'
	Reverse	5'-GCTCTCAGTCAGCAGACACTT-3'
	Probe	5'-Fam-CCGAGCTCTGCTCTGAGGCCT-BHQ1-3'
<i>ITGA2</i>	Forward	5'-TGGATTTGCGTGTGGACATC-3'
	Reverse	5'-GGCAGTTCTAGAATAGGCTTCAA-3'
	Probe	5'-Fam-TCTGGAAAACCTGGCACTAGCCCTG-BHQ1-3'
<i>ITGA5</i>	Forward	5'-GGGTGGCCTTCGGTTTACAG-3'
	Reverse	5'-GCTTTGCGAGTTGTTGAGATTC-3'
	Probe	5'-Fam-TCCCTCATCTCCGGGACACTAA-BHQ1-3'
<i>ITGB1</i>	Forward	5'-CAAGGGCAAACGTGTGAGA-3'
	Reverse	5'-TGAAGGCTCTGCACTGAACA-3'
	Probe	5'-Fam-TGTGTCAGACCTGCCTTGGTGTC-BHQ1-3'
<i>ACTB</i>	Forward	5'-TCACCCACACTGTGCCCATCTACGC-3'
	Reverse	5'-CAGCGGAACCGCTCATTGCCAATGG-3'
	Probe	5'-Fam-CAGCGGAACCGCTCATTGCCAATGG-BHQ1-3'

PICSAR, p38 inhibited cutaneous squamous cell carcinoma associated lincRNA; *ITGA2*, integrin $\alpha 2$; *ITGA5*, integrin $\alpha 5$; *ITGB1*, integrin $\beta 1$; *ACTB*, β -Actin

FIRST PERSON

First person – Minna Piipponen

First Person is a series of interviews with the first authors of a selection of papers published in Biology Open, helping early-career researchers promote themselves alongside their papers. Minna Piipponen is first author on 'Long non-coding RNA PICSAR decreases adhesion and promotes migration of squamous carcinoma cells by downregulating $\alpha 2\beta 1$ and $\alpha 5\beta 1$ integrin expression', published in *BiO*. Minna is a PhD student in the lab of Veli-Matti Kähäri at University of Turku, Finland, investigating the role of long non-coding RNAs in the progression of cutaneous squamous cell carcinoma.

What is your scientific background and the general focus of your lab?

I am a biochemist by training and I have always been interested in cell biology. Currently, I am finalizing my PhD thesis about long non-coding RNAs (lncRNAs) in cutaneous squamous cell carcinoma (cSCC). Even though it is pre-translational research, I find the clinical aspect very fascinating. The general focus of our lab is to investigate the molecular mechanisms involved in cSCC progression in order to find and characterize new biomarkers for evaluating the risk of cSCC progression and metastasis. They may also serve as novel therapeutic targets for cSCC treatment.

How would you explain the main findings of your paper to non-scientific family and friends?

It is always tricky to explain this kind of work to a non-scientific audience. To be honest, I think that even my parents do not exactly know what I am doing for work. However, people know cancer, and especially among older people someone always knows someone who has struggled with the disease. Briefly, I try to explain that the work I am doing in the lab, although at very experimental level, is aimed to improve the diagnosis of patients and hopefully bring meaningful knowledge to the scientific community, especially when designing novel cancer therapies. The main finding of the current paper was that the tumorigenic molecule that we were looking at is involved in cancer cell migration and attachment to the surrounding matrix, and this way it may regulate cancer spreading. This kind of basic research is fundamental for translating new discoveries into clinical use.

What are the potential implications of these results for your field of research?

I hope that these results bring more functional knowledge to the lncRNA research field. The field itself is relatively young and the number of new publications is growing fast. However, I feel that more detailed characterization of the molecules is needed, especially about their cellular mechanisms. When it comes to cSCC, I really think that lncRNAs (and also other non-coding RNAs) will be useful in the future as prognostic biomarkers in the clinic. Moreover, they could be potential targets for cancer therapy. Due to the heterogeneity of the tumors, it would be valuable to examine



Minna Piipponen

multiple markers simultaneously in a sample with the help of novel techniques, such as mass cytometry. It would be beneficial to include non-coding RNAs within these biomarker panels.

What has surprised you the most while conducting your research?

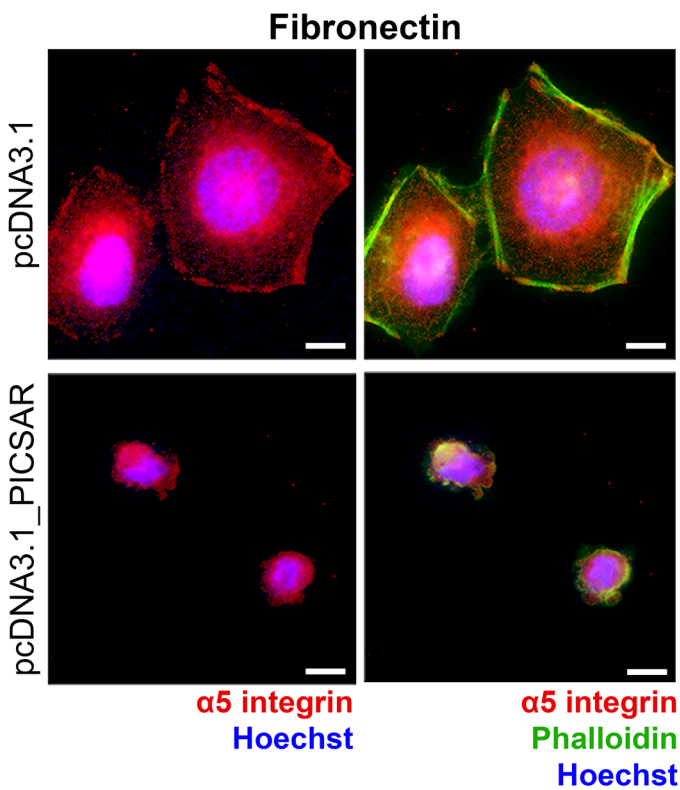
Quite early on, in the beginning of my project, it became clear to me how time consuming it is to do research. It is not just doing lab work and writing the results. It has been the most surprising to realize what it actually takes to be a researcher, a multitasking specialist in diverse fields. One is a professional lab technician, writer, translator, graphic designer, teacher, presenter, project manager, public relations person, etc., in a somewhat coordinated manner. I do not consider this as a burden, but more as a challenge and a great way to improve your skills on many levels. The longer I have been doing the research, the more confidence I have gained and realized what I am capable of. You know, as a Finn it is very hard to compliment yourself.

“It has been the most surprising to realize what it actually takes to be a researcher; a multitasking specialist in diverse fields.”

What, in your opinion, are some of your greatest achievements in your field and how has this influenced your research?

As a researcher I am still at the very early stage on my career, so in that sense I do not have much to choose from. However, I would say

Minna Piipponen's contact details: Department of Dermatology, University of Turku, Skin cancer and proteinase research group, Medisiina D, room D5056, Kiinanmyllynkatu 10, 20520 Turku, Finland.
E-mail: mmppiip@utu.fi



PICSAR overexpression in cSCC cells (lower panel) leads to significant decrease in cell adhesion and spreading.

that the greatest achievement in my field has been our first published article about a tumorigenic lncRNA that based on our results we named PICSAR. It is the first lncRNA characterized in cSCC, thus it has definitely brought new insight about lncRNA regulation in

cSCC progression. To me it has given courage to continue with the research, as there are many more lncRNAs to be discovered and investigated.

What changes do you think could improve the professional lives of early-career scientists?

I feel that increasingly people are giving credit to scientific research, and in order to continue high quality research and making new discoveries we need new scientists with fresh ideas and open minds. Briefly, I think there are a couple of things that could help the professional lives of early career scientists. First, it is not that straightforward to get financial support in the early stage of the career. This is of course something that every scientist is struggling with, however, I would wish for easier access to early career funding. Primarily, this is an issue to be solved at the governmental level. Second, I would wish for better visibility for young scientists. What bothers me the most when reading the news, especially when targeted to the public, is how only the group leader is mentioned in the context of a new discovery. Quite often the identity of the first author, who has most likely contributed the most to the research, remains unknown. I would wish for more credit to the people 'behind the scenes'.

What's next for you?

This is something I do not quite know the answer to yet. Right now I am finalizing my PhD studies and trying to plan for the mysterious life after the PhD. Preferably, I will pursue a post-doctoral position in academia. Nevertheless, I am keeping all doors open.

Reference

Piipponen, M., Heino, J., Kähäri, V. M., Nissinen, L. (2018). Long non-coding RNA PICSAR decreases adhesion and promotes migration of squamous carcinoma cells by downregulating $\alpha 2\beta 1$ and $\alpha 5\beta 1$ integrin expression. *Biol. Open* 7, bio037044.