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Research of the Expression Levels of Long Non-Coding RNAs - CASC15, LINC00346 and LIN00319 in Non-Melanotic Skin Cancers

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ABSTRACT

Background: CASC15, LINC00346 and LIN00319 LncRNAs have functional roles in cutaneous biology as in the development of skin cancers by affecting important signaling pathways involved in tumor development and progression. **Objectives:** To investigate the expression levels of long-noncoding RNAs CASC15, LINC00346 and LIN00319 in non-melanotic skin cancer (NMSC). **Subjects and methods:** included thirty patients with histopathological evidence of NMSC (basal cell carcinoma (BCC) or cutaneous squamous cell carcinoma (CSCC)) subdivided into two groups (I and II): Group I: Included 30 skin cancer tissue samples from non-melanotic skin cancer and subdivided into two subgroups: Ia: 15 skin cancer tissue samples from patients with BCC. Ib: Included 15 skin cancer tissue samples from patients with CSCC. Group II: Included 30 healthy skin marginal tissue samples from group 1 patients as controls and subdivided into two subgroups, IIa: Included 15 marginal tissue samples of healthy skin from group Ia patients. IIb: Included 15 marginal tissue samples of healthy skin from group Ib patients. Expression levels of LncRNAs CASC15, LINC00346 and LINC00319 were measured in cancerous and healthy skin samples by quantitative reverse transcription-polymerase chain reaction. **Results:** Statistically significant increase in the expression levels of CASC15, LINC00346 and LINC00319 LncRNAs in cancerous skin tissue of NMSC patients compared to their controls.

Conclusion: In NMSC skin tissue samples, CASC15, LINC00346 and LINC00319 LncRNAs are upregulated; these findings suggested that knocking down these LncRNAs could inhibit tumour growth and migration.

Keywords: CASC15, LINC00346, LINC00319, long-noncoding RNA and non-melanotic skin cancers.

INTRODUCTION

Non-melanoma skin cancers (NMSCs) account for approximately 20% of all malignancies and their incidence is steadily increasing. NMSC consists of cutaneous squamous cell carcinoma (CSCC) and basal cell carcinoma (BCC) characterized by malignant growth of keratinocytes in epithelial layer. NMSC is the most common type of cancer worldwide, and its incidence is notably increasing partly due to advanced surveillance and aging populations [(1)].

Basal cell carcinoma (BCC) is the most frequently diagnosed malignancy with increasing annual incidence. Also, it is the most common malignant epithelial tumor worldwide that statistically constituting 80% of keratinocyte cancers [(2)].

BCC is initiated from stem cell populations found in the follicle bulge and basal layer of the interfollicular epidermis. Its growth and development are delayed. It damages the adjacent tissues through partial invasion, with very little migration. The standard treatment for BCC is excision or Mohs surgery [(3)].

CSCC is the second most frequent cancer in humans and its incidence continues to rise. Although CSCC usually displays benign clinical behavior, it can be both locally invasive and metastatic. Ten-year survival after surgery exceeds 90% while it drops dramatically when metastases occur. CSCC arises from the malignant proliferation of epidermal keratinocytes. Chronic sun exposure is the most important and well-known environmental factor associated with CSCC [(4)].

Besides UV exposure, comorbidities like diabetes, rheumatoid arthritis, and psoriasis may also influence CSCC development [(5)]. Cancer-associated susceptibility 15 gene (CASC15), a newly discovered lncRNA which is located at 6p22.3**,** is an oncogenic factor that has been identified in several types of cancer, such as tongue squamous cell carcinoma, oral squamous cell carcinoma, melanoma and cervical cancer [(6)].

CASC15 stimulates cell proliferation and suppresses cell apoptosis by activating WNT/β-catenin signaling pathway [(7)]. lncRNA LINC00346 is specifically overexpressed by CSCC cells. LINC00346 is regulated by p53.LINC00346 regulates the expression and activity of STAT3, which in turn up-regulates the expression of matrix metalloproteinases (MMPs) MMP-1, MMP-3, MMP-10, and MMP-13 and promotes invasion of CSCC cells so it was named p53 regulated carcinoma-associated STAT3 activating long intergenic non-protein coding transcript (PRECSIT) [(8)].

Long noncoding RNA (lncRNA) LINC00319 is located on chromosome 21q22.3. It has a transcript length of 2901 nucleotides. its expression has been identified to be upregulated in cutaneous squamous cell carcinoma with studies suggesting its relationship with poor prognosis is due to its ability to promote cell proliferation and invasion [(9)].

The objectives: of this study was to investigate the expression levels of long-noncoding RNAs CASC15, LINC00346 and LIN00319 in non-melanotic skin cancer (NMSC).

SUBJECTS AND METHODS

This study was carried out by cooperation between Medical Biochemistry and Molecular Biology

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Department and Pathology Department, Faculty of Medicine, Menoufia University. It included 30 subjects with histopathological proof of non-melanoma skin cancer (BCC or CSCC) selected from Plastic Surgery Department, Menoufia University Hospital in the period from June 2023 to March 2024. The patients were divided as follow: Group I: Included 30 skin cancer tissue samples from patients with non-melanotic skin cancer, it was divided into 2 subgroups: Ia: Included 15 skin cancer tissue samples from patients diagnosed as BCC. Ib: Included 15 skin cancer tissue samples from patients diagnosed as CSCC. And Group II: Included 30 healthy skin marginal tissue samples taken from group 1 patients as control. It was divided into 2 subgroups: IIa: Included 15 healthy skin marginal tissue samples taken from group Ia patients (BCC). IIb: Included 15 healthy skin marginal tissue samples taken from group Ib patients (CSCC).

METHODS

All studied participants were subjected to full history taking, complete general examination, neck ultrasound for lymph node metastasis in cases of CSCC, preoperative incisional biopsy for histopathological examination, sampling (Tissue samples from cancerous skin tissue and adjacent healthy marginal tissue was taken for RNA extraction using **miRNeasy Mini Kit,** Cat 217004, Qiagen, USA) and Quantification of expression levels of LncRNAs CASC15, LINC00346 and LINC00319 by quantitative reverse transcription-polymerase chain reaction (RT-PCR)**.**

Estimation of gene expressions of LncRNAs CASC15, LINC00346 and LINC00319:

RNA isolation from tissue (miRNeasy Mini Kit, Cat 217004, Qiagen, USA)

Tissue was homogenized in the presence of a highly denaturing Qiazol reagent, which immediately inactivates RNases to ensure purification of intact RNA. Ethanol was added to provide appropriate binding conditions, and the sample was then applied to RNease Spin IIC column, where the total RNA bound to the silica-based membrane and contaminants were efficiently washed away. High-quality RNA was then eluted in 50µl RNase free water.

Assuring RNA quantity and purity

The concentration of extracted RNA was determined by measuring its absorbance at 260 nm (A260) using nanophotometer N-60. The ratio between the absorbance value at 260 and 280 nm (A260 / A280) gives an estimate of RNA purity. (A260 / A280) ratio between 1.8 and 2.1 was accepted. If the purity was lower than 1.8, it required re-extraction. A 260/280 ratio of our RNA extract was between 1.8 and 2.2, which is accepted.

First Step - PCR: cDNA Synthesis (RT- Step) (Revert Aid First Strand cDNA Synthesis Kit, Thermo Fisher Scientific Inc., USA) RT Primer Mix ensures cDNA synthesis from all regions of RNA transcripts, even from 5' regions. This allows high yields of cDNA template for real-time PCR analysis regardless of where the target region is located on the transcript.

Second Step-PCR: Amplification of cDNA for detection of expression of LncRNAs CASC15, LINC00346 and LINC00319 (Thermo Scientific Maxima SYBR Green, ROX Master Mix (2X), #K0221, Thermo Fisher Scientific, USA). SYBR Green II binds all double-stranded DNA molecules, emitting a fluorescent signal on binding. The presence of ROX passive reference dye in real-time PCR compensates for non-PCR–related variations in fluorescence detection. Fluorescence from ROX dye does not change during the course of real-time PCR but provides a stable baseline to which PCR-related fluorescent signals are normalized. Thus, ROX dye compensates for differences in fluorescence detection between wells due to slight variations in reaction volume or to differences in well position. The following primers were used ***(https://www.ncbi.nlm.nih.gov/tools/primer-blast/)***.

Forward and reverse primers for LncRNA CASC15, 5'-CTTTGTCTGCTCCGGGACTT -3' and 5'-TTAAGGGACATTTCCCCCGC -3'; forward and reverse primers for LINC00346, 5'-CGAGGGTTGAACATTGTTGTGAC -3' and 5'-CCACAGCTCCACCACTAGAC -3'; forward and reverse primers for LINC00319, 5'-GGAAGCCGGATAAGCACCTC -3' and 5'-GCTACGCTGCAGTCACAAAC -3'; and forward and reverse primers for GAPDH (endogenous control), 5'-CCACTCCTCCACCTTTGAC-3' and 5'-ACCCTGTTGCTGTAGCCA-3'.

The following conditions were applied to conduct PCRs: Initial denaturation: 1 cycle for 10 minutes at 95°C, denaturation: 40 cycles each 15 seconds at temperature 95°C, Annealing/ Extension: 40 cycles each 60 seconds at temperature 60°C.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**. (**Armonk, NY: IBM Corp**)**. Qualitative data were described using number and percent. The Shapiro-Wilk test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation (SD), median and interquartile range (IQR). The significance of the obtained results was judged at the 0.05% level. The used tests were Chi-square test, Fisher's Exact, Student t-test, Wilcoxon signed ranks test and Spearman coefficient.

Ethical Approval:

The research was permitted through the Ethics Committee of Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Menoufia University, Egypt. All participants gave written informed consent before enrolment. The study adhered to the Helsinki Declaration throughout its execution.

RESULTS

There was significant statistical difference between BCC patients and CSCC patients regarding DM. 40% of CSCC patients in this study had DM. There was no significant statistical difference between BCC patients and CSCC patients regarding sun exposure, smoking, number, color and consistency of lesion, TLIs, LVI and perineural invasion and N/ C ratio **(Table 1).**

Table (1): Comparison between group Ia (BCC cases) and Ib (CSCC cases) according to demographic and clinical data.

	Group Ia (BCC) (n = 15)		Group Ib (CSCC) (n = 15)		Test of Sig.	P
	No.	%	No.	%		
Gender						
Male	13	86.7	11	73.3	χ^2=	> 0.05
Female	2	13.3	4	26.7	0.833	
Age (years)						
Min. – Max.	37.0 – 75.0		25.0 – 80.0		t=	> 0.05
Mean ± SD.	63.13 ± 10.15		63.60 ± 13.36		0.108	
Median (IQR)	63.0 (59.0 – 71.0)		66.0 (61.0 – 71.50)			
Sun exposure	13	86.7	12	80.0	0.240	> 0.05
Family history	0	0.0	0	0.0	–	–
Smoking	2	13.3	2	13.3	0.000	> 0.05
Comorbidities						
HTN	8	53.3	7	46.7	0.133	> 0.05
Virus C	2	13.3	4	26.7	0.833	> 0.05
DM	0	0.0	6	40.0	7.500*	< 0.05*
Rt fibroadenoma resected	1	6.7	0	0.0	1.034	> 0.05
Number of lesions						
Single	13	86.7	9	60.0	FET= 3.774	> 0.05
Two	2	13.3	3	20.0		
Three	0	0.0	1	6.7		
Four	0	0.0	1	6.7		
Multiple	0	0.0	1	6.7		
Color of lesion						
Black	1	6.7	0	0.0	FET= 4.274	> 0.05
Brown	3	20.0	0	0.0		
Gray	3	20.0	5	33.3		
Greyish white	8	53.3	10	66.7		
Consistency of lesion						
Soft	11	73.3	6	40.0	FET= 4.203	> 0.05
Rubbery	2	13.3	2	13.3		
Firm	2	13.3	7	46.7		
TILs	2	13.3	0	0.0	χ^2= 2.143	> 0.05
LVI	0	0.0	1	6.7	χ^2=1.034	> 0.05
Perineural invasion	0	0.0	0	0.0	–	–
Increase N/C						
No	10	66.7	10	66.7	χ^2= 0.000	> 0.05
Yes	5	33.3	5	33.3		

BCC: basal cell carcinoma, CSCC: cutaneous squamous cell carcinoma, DM: Diabetes mellitus, TILs: Tumor infiltrating lymphocytes, HTN: Hypertension, N/C: Nucleocytoplasmic ratio, LVI: Lymph vascular invasion, IQR: Interquartile range, SD: Standard deviation, t: Student t-test, χ^2: Chi square test, FE: Fisher Exact test, *: Statistically significant at $p \leq 0.05$.

In this study the majority of CSCC patients were with grade II, T1 stage and N0 stage. Nodal metastasis was present in about 20% of CSCC patients. The majority of CSCC patients (80%) have no involved lymph nodes (free) **(Table 2).**

Table (2): Distribution of the studied cases according to tumor characteristics in group Ib (CSCC cases) (n = 15)

	No.	%
Grade		
I	3	20.0
II	9	60.0
III	3	20.0
T stage		
T1	8	53.3
T2	6	40.0
T3	1	6.7
N stage		
N0	12	80.0
N1	1	6.7
N2	2	13.3
M stage (M0)	15	1.00
Neck US		
Free	12	80.0
Cervical LN	1	6.67
Subcentimetric, upper and post cervical	1	6.67
Submental, submandibular, upper deep cervical	1	6.67

There was a significant statistical increase of the expression levels of CASC15, LINC00346 and LINC00319 long noncoding RNAs in skin tissue samples of BCC patients compared with their controls **(Table 3)**.

Table (3): Comparison of the expression levels of studied long noncoding RNAs in skin tissue samples between groups Ia and IIa.

	Group Ia (n=15)	Group IIa (n=15)	Z	p
CASC15				
Min. – Max.	0.16 – 15.50	0.0 – 1.82		
Mean ± SD.	2.11 ± 3.90	0.61 ± 0.54	2.272	< 0.05*
Median (IQR)	0.67 (0.29 – 1.84)	0.54 (0.20 – 1.0)		
LINC00346				
Min. – Max.	0.32 – 3.93	0.21 – 1.88		
Mean ± SD.	1.45 ± 1.08	0.81 ± 0.36	2.101*	< 0.05*
Median (IQR)	1.0(0.75 – 2.04)	0.81(0.63 – 0.88)		
LINC00319				
Min. – Max.	0.01 – 3.95	0.01 – 1.09		
Mean ± SD.	1.18 ± 1.15	0.55 ± 0.36	2.158*	< 0.05*
Median (IQR)	0.71(0.38 – 1.98)	0.54(0.24 – 0.87)		

CASC15: malignancy susceptibility 15, LINC00346: long intergenic noncoding 00346, LINC00319: long intergenic noncoding 00319, IQR: Interquartile range, SD: Standard deviation, Z: Wilcoxon signed ranks test, *: Statistically significant at $p \leq 0.05$

There was a significant statistical increase of the expression levels of CASC15, LINC00346 and LINC00319 long noncoding RNAs in skin tissue samples of CSCC patients compared with their controls **(Table 4)**.

Table (4): Comparison of the expression levels of studied long noncoding RNAs in skin tissue samples between groups Ib and IIb.

	group Ib (n=15)	group IIb (n=15)	Z	p
CASC15				
Min. – Max.	0.21 – 3.76	0.14 – 1.41		
Mean ± SD.	1.28 ± 0.90	0.75 ± 0.37	2.215*	< 0.05*
Median (IQR)	1.07 (0.81 – 1.57)	0.94 (0.39 – 1.0)		
LINC00346				
Min. – Max.	0.39 – 3.99	0.41 – 1.58		
Mean ± SD.	1.51 ± 0.89	0.96 ± 0.35	2.442*	< 0.05*
Median (IQR)	1.24 (1.06 – 1.68)	0.92 (0.72 – 1.17)		
LINC00319				
Min. – Max.	0.04 – 3.15	0.26 – 1.67		
Mean ± SD.	1.32 ± 0.90	0.87 ± 0.41	2.272*	< 0.05*
Median (IQR)	1.25 (0.48 – 1.95)	0.89 (0.51 – 1.0)		

CASC15: malignancy susceptibility 15, LINC00346: long intergenic noncoding 00346, LINC00319: long intergenic noncoding 00319, IQR: Interquartile range, SD: Standard deviation, Z: Wilcoxon signed ranks test, *: Statistically significant at $p \leq 0.05$.

There was significant positive correlation between expression levels of the three studied LNCRNAs in skin tissue samples of BCC patients **(Table 5).**

Table (5): Correlation coefficient of the expression levels of studied long noncoding RNAs in tissue samples of group Ia (BCC) (n=15)

	CASC15		LINC00346		LINC00319	
	r_s	p	r_s	p	r_s	p
CASC15			0.633	< 0.05*	0.559	< 0.05*
LINC00346					0.664	< 0.05*
LINC00319						

LINC00346: long intergenic noncoding 00346, LINC00319: long intergenic noncoding 00319, CASC15: malignancy susceptibility 15, r_s: Spearman coefficient, *: Statistically significant at $p \leq 0.05$

There was a significant positive correlation between expression levels of LINC00319 in skin tissue samples of CSCC patients and age. There was a significant positive correlation between expression levels of CASC15 in skin tissue samples of CSCC patients and T-staging. There was a significant positive correlation between expression levels of LINC00346 and T-staging in skin tissue samples of CSCC patients **(Table 6)**.

Table (6): Correlation coefficient between studied LNCRNAs in skin tissue samples and each of age, staging and grading of tumor in group Ib (CSCC) (n=15)

	CASC15		LINC00346		LINC00319	
	r_s	p	r_s	p	r_s	p
CASC15			0.786	< 0.05*	0.521	< 0.05*
LINC00346					0.636	< 0.05*
LINC00319						
Age (years)	-0.107	0.703	-0.004	> 0.05	0.525	< 0.05*
Grading	-0.293	0.290	-0.439	> 0.05	0.0	> 0.05
T stage	0.606	0.017*	0.606	< 0.05*	0.423	> 0.05
N stage	-0.079	0.779	0.069	> 0.05	0.327	> 0.05

LINC00346: long intergenic noncoding 00346, LINC00319: long intergenic noncoding 00319, CASC15: malignancy susceptibility 15, r_s: Spearman coefficient, *: Statistically significant at $p \leq 0.05$.

DISCUSSION

Non-melanoma skin carcinoma (NMSC) is the most common malignant tumor in the population, with a steadily increasing incidence due to an aging population and sun exposure. The two main subtypes of NMSC are basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (CSCC) [(1)]. Non-coding RNAs (ncRNAs) have a significant regulatory role in the pathogenesis of skin cancer, LncRNAs are remarkably adaptable, acting as scaffolding, guides, or decoys to modify key signaling pathways (i.e., the WNT/β-catenin pathway) and gene expression [(7)]. In this study we determined the role of long noncoding ribonucleic acids: CASC15, LINC00346 and LINC00319 in non-melanotic skin cancers, showing their expression levels in these tissues and adjacent non-tumorous tissues using RT-PCR analysis.

In this study, there was no significant statistical difference between BCC patients and CSCC patients regarding age. Most of patients in this study were old age (above 60 years) with mean (63.13 ± 10.15) in BCC and (63.60 ± 13.36) in CSCC. **Özgür and colleagues** [(10)] reported that skin cancers generally develop in the elderly population, the mean age of patients was 71.4±11.05 years. Similarly, **Ragi and colleagues** [(11)] reported in their clinical study a mean age of 71.8 years in skin cancer patients. The main reason why the disease is more common among the elderly is their cumulative sun exposure over the years. In this study, there was no significant statistical difference between BCC patients and CSCC patients regarding sun exposure and smoking in spite of 86.7% of BCC patients and 80% of CSCC patients in the present study were chronically exposed to sun and 13.3% of both BCC and CSCC patients were smoking. Higher UV exposure has been reported to increase risk for BCC and CSCC.

However, the increased relative risk of keratinocyte carcinomas with higher levels of UV exposure is greater for CSCC (2.05; 95% CI, 1.54- 2.73) than for BCC (1.30; 95% CI, 1.18-1.43). The differential effect of UV exposure on CSCC and BCC may partially account for the generally lower BCC:CSCC ratios seen in more southern regions [(12)].

Song and colleagues [(13)] reported that "ever" smokers were at slightly increased risk of BCC (odd ratio (OR) 1.02, 95% confidence interval (CI) 1.00–1.04) and CSCC (OR 1.08, 95% CI 1.01–1.15) relative to never smokers. **Leonardi-Bee *et al.*** [(14)] reported a significant 50% increase in the risk of CSCC to ever smokers, but no association was seen with BCC (OR, 0.95, 95% CI 0.82–1.09).

More recently, the United Kingdom Million Women Study found that in individuals who currently smoke compared to those who have never smoked, there was an elevated incidence of SCC (RR 1.22, 95% CI 1.15–1.31), while the incidence of BCC decreased (RR 0.80, 95% CI 0.78–0.82) [(15)]. **Dusingize and colleagues** [(16)] supported the findings of observational studies linking smoking to lower risks of BCC. But found no evidence that smoking is associated with an elevated risk of CSCC; indeed, their results were most consistent with a decreased risk, similar to BCC.

In the present study, there was a significant statistical difference between BCC patients and CSCC patients regarding DM. 40% of CSCC patients in this study had DM, which is considered a risk factor for developing CSCC. These results are in accordance with [(17)].

Accumulating evidence supports the hypothesis that DM, in addition to UV radiation, may be involved in the carcinogenesis of CSCC. Patients with DM have an increased susceptibility to infections and typically exhibit chronic ulcers in the lower extremities. Cases of CSCC arising in long-standing foot ulcers of diabetic patients have been reported as a complication of DM [(17,18)]. Marjolin's ulcer is a rare but aggressive type of CSCC cancer and arises in chronic ulcers caused by vascular insufficiency, diabetic neuropathy, pressure or hemoglobinopathy or in scar tissue [(8)]. Also, **Larijani and colleagues** [(19)] reported in their study that in chronic, nonhealing diabetic foot ulcers with the presence of old age and sun exposure risk factors, diagnosis of SCC should be considered and ruled out. We found in our study that the majority of CSCC patients were with grade II, T1 stage and N0 stage. Lymph nodes metastases from CSCC were present in about 20% of CSCC patients. The majority of CSCC patients (80%) in this study had no involved lymph nodes (free), which is supported by [(20)] who reported that the majority of CSCC tends to carry a favorable prognosis with high rates of local control and rates of distant metastasis as low as 2-3%. Overall, 5-20% of patients present with nodal metastases from CSCC.

In this study, we found a significant statistical increase in the expression levels of CASC15 long noncoding RNA in skin tissue samples of BCC patients compared with their controls. These results are in accordance with [(21)] who reported that CASC15 is upregulated in BCC. Our results are also supported by **Sheng and Wei** [(7)] who reported that CASC15 stimulates cell proliferation and suppresses cell apoptosis by activating WNT/β-catenin signaling pathway and **Noubissi and colleagues** [(22)] who reported that this pathway is implicated in basal cell carcinoma development, and plays a critical role in patterning and cell proliferation of embryonic and adult tissues.

The WNT signaling pathway stimulates the transcriptional output of Hh signaling, activation of Hh appears to be a key driver of BCC development, WNT signaling induces the expression of RNA-coding region determinant binding protein (CRD-BP) or insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1), which in turn binds and stabilizes GLI1 mRNA, thereby upregulating GLI1 expression and transcriptional activity which leads to constant activation of HH and uncontrolled canonical cell proliferation [(23)].

Even though recent studies have identified CASC15 as an oncogene in several types of cancer, such as tongue squamous cell carcinoma, oral squamous cell carcinoma, melanoma and cervical cancer[6],

its role in CSCC remains elusive. To investigate the biological role of CASC15 in CSCC, we carried out qRT-PCR analysis to determine its expression in 15 paired CSCC tissues and adjacent noncancerous tissues, and we found that CASC15 was significantly upregulated in CSCC tissues compared with matched nontumorous skin tissues. Our results may be supported by [7] who reported that CASC15 stimulates cell proliferation and suppresses cell apoptosis by activating WNT/β-catenin signaling pathway and [24] who reported that WNT/β-catenin signaling is essential for HPV-driven CSCC initiation and progression as well as for maintaining the cancer stem cell niche. HPV-driven CSCC is the most common cancer in immunosuppressed organ transplant recipients (OTR), up to 50% of those patients develop CSCCs within 10 years after transplantation [25].

Recent studies have found that LINC00346 and LINC00319 LncRNAs are upregulated in CSCC [8,9] but their role in BCC has not been identified. LINC00346 up-regulates the expression of MMP-1, MMP-3, MMP-10, and MMP-13 [8]. LINC00319 upregulates expression of MMP-2, MMP-9, and markers for epithelial–mesenchymal transition, E-cadherin, and vimentin [21]. MMPs are involved in BCC development and tumor growth. MMP-1 and MMP-2 secreted by fibroblasts facilitates tumor growth. MMP-13 secreted by fibroblasts and tumor cells promotes tumor angiogenesis. MMP-9 secreted by inflammatory cells activates BCC cells to secrete VEGF, which in turn promotes angiogenesis [26]. LINC00346 regulates the expression and activity of STAT3, which in turn up-regulates the expression of matrix metalloproteinases (MMPs) MMP-1, MMP-3, MMP-10, and MMP-13 [8]. STAT3 signaling pathway is involved in BCC carcinogenesis, during the development of ultraviolet (UV) B skin tumors, STAT3 plays a critical role in both survival and proliferation of keratinocytes [27].

We also found in our study a significant statistical increase of the expression levels of LINC00346 long noncoding RNA in skin tissue samples of CSCC patients compared with their controls. These results are in accordance with **Piipponen and colleagues** [8] who reported in their study that there is a significant up-regulation of PRECSIT was detected in CSCC cell lines compared with normal skin tissue samples. Also, **Piipponen and colleagues** [28] identified PRECSIT (LINC00346) as one of the most up-regulated lncRNAs in CSCC cells with very low expression in normal human epidermal keratinocytes (NHEKs) based on RNA-seq. **Zhou and colleagues** [29] have found that Jak-STAT signaling is one of the most potential targets for PRECSIT. In addition, significant down-regulation of STAT3 mRNA and protein levels after PRECSIT knockdown was detected resulting in potent reduction in the levels of activated STAT3, indicating that the effect of PRECSIT is mediated via STAT3 signaling in CSCC cells.

In our research we found a significant statistical increase of the expression levels of LINC00319 long noncoding RNA in skin tissue samples of CSCC patients compared with their controls, these results are in accordance with [9] who reported that LINC00319 expression has been identified to be upregulated in cutaneous squamous cell carcinoma with studies suggesting its relationship with poor prognosis is due to its ability to promote cell proliferation and invasion. Also, **Li and colleagues** [30] carried out qRT-PCR analysis to determine LINC00319 expression levels in 60 paired CSCC tissues and adjacent noncancerous skin tissue samples and found that LINC00319 was significantly upregulated in CSCC tissues compared with matched non-tumorous tissues. In addition, **Piipponen and colleagues** [21] reported that LINC00319 is upregulated in CSCC as it increases CSCC cell growth, migration, and invasion and suppresses apoptosis by upregulating cyclin-dependent kinase 3 via miR-1207-5p decoy. LINC00319 could competitively bind to miR-1207-5p in CSCC cells.

In addition, CDK3 (known as a member of the CDKs family, is closely associated with G0/G1 and G1/S cell cycle transitions.) was identified as a direct target of miR-1207-5p in CSCC cells. Furthermore, LINC00319 was found to modulate CDK3 expression by sponging miR-1207-5p in CSCC cells [30].

We found no significant statistical difference between BCC patients and CSCC patients regarding the expression levels of CASC15, LINC00346 and LINC00319 long noncoding RNAs in skin tissue samples. As we found that the three studied LncRNAs are upregulated in both BCC and CSCC cancerous skin tissue samples. However, we found a significant positive correlation between expression levels of the three studied LNCRNAs in cancerous skin tissue samples of BCC and CSCC patients.

In this study we found a significant positive correlation between expression levels of CASC15 in skin tissue samples of CSCC patients and T-staging. Non-significant negative correlations existed with age, grading and N-staging. This indicates that CASC15 may be involved in tumor growth in CSCC patients. We also found a significant positive correlation between expression levels of LINC00346 and T-staging in skin tissue samples of CSCC patients. Non-significant negative correlations existed with each of age and grading and non-significant positive correlations existed with N-staging. **Piipponen and colleagues** [8] found that the percentage of PRECSIT-positive cells was significantly higher in invasive CSCC than in noninvasive CSCC precursor lesions as AK, and higher in AK than normal skin. In this study we found a significant positive correlation between expression levels of LINC00319 in skin tissue samples of group CSCC patients and age. In contrast **Li and colleagues**

[30] found a non-significant positive correlation between expression levels of LINC00319 in skin tissue samples of CSCC patients and age, this may be explained with the differences in sample size as their study was carried out on 60 paired tumorous tissues and matched nontumorous tissues were collected from CSCC patients. In this study there were non-significant negative correlations existed between expression levels of LINC00319 in skin tissue samples of CSCC patients with each of grading, T-staging and N-staging. However, **Li and colleagues** [30] reported that increased expression of LINC00319 was associated with larger tumor size, advanced TNM stage, and lymphovascular invasion and implies a potential link with poor prognosis and reflects CSCC progression.

CONCLUSION

The three studied LncRNAs are upregulated in BCC and CSCC skin tissue samples. There is significant positive correlation between expression levels of the three studied LNCRNAs in skin tissue samples of BCC and CSCC patients. Knockdown of these LncRNAs could inhibit tumor proliferation and migration and can be used as diagnostic biomarkers for BCC and CSCC.

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