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

Naglaa M. Ghanayem[1], Yasser A. Elghobashy[1], Nahla M. Badr[2,4], Mohamed Salah Abo El Hoda[3], Mariam S. Gaafar[1,*], Manal Abd El-Monem Ellaithy[1]

Departments of [1]Medical Biochemistry and Molecular Biology, [2]Pathology, [3]Plastic and Reconstructive Surgery, Faculty of Medicine, Menoufia University, Egypt and [4]Department of Pathology, Al-Ryada University for Science and Technology, Egypt

*** Corresponding author:** Mariam S. Gaafar, **Email:** mariam.gaafar96@gmail.com, **Mobile No.: +201068665217**

ABSTRACT

Background: CASC15, LINC00346 and LIN00319 LncRNAs have functional roles in cutaneous biology as in the progress of skin tumors 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:** This study 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 cases 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 through quantitative reverse transcription-PCR. **Results:** Statistically significant rise 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 constitute more than twenty percent of all malignancies, with their incidence steadily rising. NMSC comprises cutaneous squamous cell carcinoma and basal cell carcinoma, both defined by the malignant growth of keratinocytes inside the epithelial layer. Non-melanoma skin cancer is the most prevalent tumor globally, with its frequency rising significantly, partially because of enhanced surveillance and an aging population [(1)]. BCC is the most often detected tumor, with a rising yearly frequency. Moreover, it is the most prevalent malignant epithelial neoplasm globally, accounting for eighty percent of keratinocyte tumors [(2)]. Basal cell carcinoma originates from stem cell populations located in the basal layer and the follicle bulge of the interfollicular epidermis. Its development and growth are delayed. It causes damage on the adjacent tissues via partial invasion, exhibiting minimal migration. The standard management for BCC is excision or Mohs operation [(3)].

CSCC is the 2nd most prevalent tumor in humans, and its frequency is on growing. Despite its often benign clinical behavior, CSCC can exhibit local invasiveness and metastasis. Ten-year survival rates post-surgery surpass ninety percent but decline significantly in the presence of metastases. CSCC originates from the malignant growth of epidermal keratinocytes. Prolonged sun exposure is the 1ry and most recognized environmental factor related to CSCC [(4)].

In addition to UV exposure, comorbidities such as rheumatoid arthritis, psoriasis, and diabetes may also affect the development of CSCC [(5)]. The Cancer-associated susceptibility 15 genes (CASC15), a recently revealed long non-coding RNA located at 6p22.3, is an oncogenic factor involved in various cancers, comprising oral squamous cell carcinoma, tongue squamous cell carcinoma, cervical cancer, and melanoma [(6)].

CASC15 inhibits apoptosis and promotes cellular proliferation through stimulating the WNT/β-catenin signaling pathway [(7)]. lncRNA LINC00346 is exclusively overexpressed in CSCC. LINC00346 is controlled through p53. LINC00346 modulates the expression in addition to activity of STAT3, which subsequently enhances the expression of matrix metalloproteinases (MMPs) matrix metalloproteinases -3, matrix metalloproteinases -1, matrix metalloproteinases -13, and matrix metalloproteinases -10, thereby facilitating the invasion of CSCC cells; hence, it is designated as p53 regulated carcinoma-associated STAT3 triggering long intergenic non-protein coding transcript (PRECSIT) [(8)].

The long noncoding RNA (lncRNA) LINC00319 is situated on chromosome 21q22.3. The transcript length is 2901 nucleotides. Its expression has been found to be elevated in CSCC, with research indicating that its association with bad prognosis is attributable to its ability to enhance cell invasion and proliferation [(9)].

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

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SUBJECTS AND METHODS

This research has been performed by cooperation between Medical Biochemistry and Molecular Biology Department and Pathology Department, Faculty of Medicine, Menoufia University. Thirty cases with histologically confirmed diagnoses of non-melanoma skin cancer (CSCC and BCC) were included **(Figure 1 and 2).** Histopathological assessment was conducted on hematoxylin and eosin (H&E)-stained sections to verify the diagnosis and assess key tumor parameters. Cutaneous squamous cell carcinoma (CSCC) is distinguished by nests or sheets of malignant squamous cells invading the dermis. These cells frequently show keratinization, intercellular bridges, nuclear pleomorphism, and elevated mitotic activity **(Figure 1)**. CSCC histologic grade was assessed based on tumor differentiation [(10)] (**Figure 1a, 1b, 1c)**, perineural invasion (PNI) **(Figure 1d)** was determined by evidence of tumor infiltration along or around nerve structures [(11)]**.** The extent of tumor-infiltrating lymphocytes (TILs) was quantified as the percentage of stromal area occupied by TILs [(12)] **(Figure 1e),** and lymph vascular invasion (LVI) defined by the existence of tumor emboli within vascular or lymphatic channels [(13)] **(Figure 1f).** The histopathological features of basal cell carcinoma (BCC) include neoplastic basaloid cells with peripheral palisading and abnormal mitotic figures, which are characteristic findings aiding in the confirmation of BCC diagnosis (**Figure 2).** Cases were selected from Plastic Surgery Department, Menoufia University Hospital in the duration 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 cases 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).

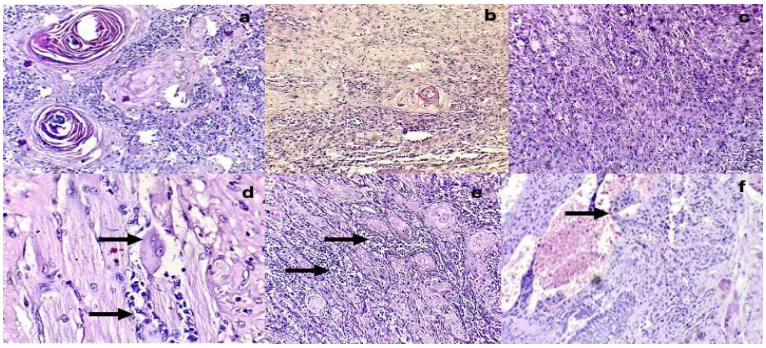

Figure 1; Histopathology of CSCC cases, a: A case of well-differentiated CSCC (grade I) (H&E x200), b: A case of moderately differentiated CSCC (grade II) (H&E x200). c: A case of poorly differentiated CSCC (grade III) (H&E x200). d: A case of CSCC showing perineural invasion (H&E x400) (black arrows). e; A case of CSCC showing tumor infiltrating lymphocytes around the malignant cell nests (H&E x100) (black arrows). f: A case of CSCC showing lymph-vascular invasion (LVI) by the tumor nests (H&E x200) (black arrows)

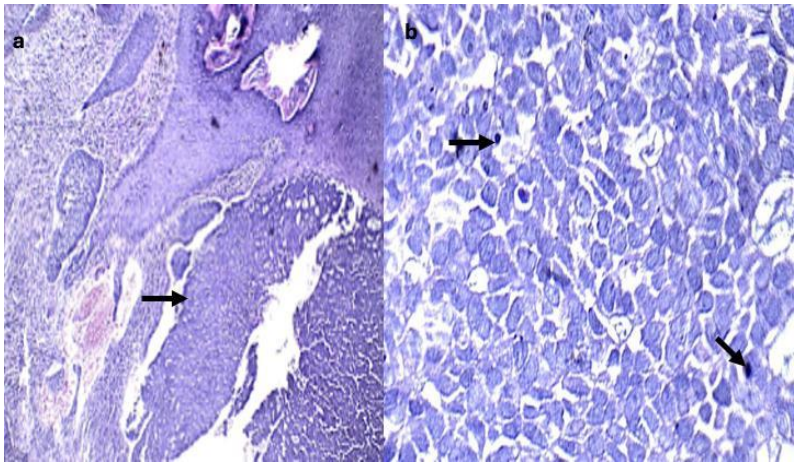

Figure 2; Histopathology of BCC cases. a: A case of BCC showing invasion by neoplastic growth formed of malignant basaloid cells with peripheral palisading (H&E x200) (black arrows), b: A case of BCC showing abnormal mitotic figures (H&E x400) (black arrows).

METHODS

All studied participants have been exposed 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 has been homogenized using a powerful denaturing Qiazol reagent, which promptly inactivates RNases to guarantee the purification of intact RNA. Ethanol has been added to establish optimal binding conditions, after which the sample has been placed to the RNease Spin IIC column, allowing the entire RNA to adhere to the silica-based membrane while impurities were effectively removed. Subsequently, high-quality RNA has been eluted in fifty milliliters of RNase-free water.

Assuring RNA quantity and purity

The concentration of extracted RNA has been measured through assessing its absorbance at 260 nanometers (A260) with a nanophotometer N-60. The absorbance ratio at 260 nanometers to that at 280 nanometers (A260 / A280) provides an estimation of RNA purity. A (A260 / A280) ratio varying from 1.8 to 2.1 was considered acceptable. If the purity fell below 1.8, re-extraction was necessary. The A260/A280 ratio of our RNA extract ranged from 1.8 to 2.2, which was deemed acceptable.

First Step - PCR: cDNA Synthesis (RT- Step) (Revert Aid 1st Strand cDNA Synthesis Kit, Thermo Fisher Scientific Inc., USA) RT Primer Mix ensures synthesis of cDNA from all areas of RNA transcripts, even from 5' areas. This permits elevated produces of cDNA template for real-time PCR examination in spite of where the target area is situated 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). All double-stranded DNA molecules can be bound by SYBR Green II, which then releases a fluorescent signal. In real-time polymerase chain reaction, ROX passive reference dye reduces fluorescence recognition variations unrelated to PCR. The fluorescence of ROX dye remains constant throughout real-time PCR, serving as a steady baseline for normalizing PCR-related fluorescent data. Consequently, ROX dye compensates for variances in fluorescence identification among wells caused by small differences in reaction volume or variances in well position. The subsequent primers have been utilized.

(https://www.ncbi.nlm.nih.gov/tools/primer-blast/).

Reverse and Forward primers for lncrna CASC15, 5'-CTTTGTCTGCTCCGGGACTT -3' reverse and 5'-TTAAGGGACATTTCCCCCGC -3'; and forward primers for LINC00346, 5'-CGAGGGTTGAACATTGTTGTGAC -3' and 5'-CCACAGCTCCACCACTAGAC -3'; reverse and forward primers for LINC00319, 5'-GGAAGCCGGATAAGCACCTC -3' and 5'-GCTACGCTGCAGTCACAAAC -3'; and reverse and forward 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 ninety-five degrees Celsius, denaturation: forty cycles each fifteen seconds at temperature 95°C, Annealing/ Extension: 40 cycles each 60 seconds at temperature 60°C.

Statistical analysis

The information has been fed into the computer and examined applying IBM SPSS software version 20.0 (Armonk, NY: IBM Corp). Qualitative information has been defined utilizing percentages and numbers. The Shapiro-Wilk test has been utilized to assess the normality of the distribution. The quantitative information has been defined by range (maximum and minimum), mean, standard deviation (SD), median and interquartile range (IQR). The significance of the attained outcomes has been judged at the 0.05% level. The applied tests were Fisher's Exact, Chi-square test, Wilcoxon signed ranks test, Student t-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. A written informed consent was obtained from each and every participant before to enrollment. The research adhered to the Helsinki Declaration throughout its execution.

RESULTS

A significant statistical variance has been observed between BCC patients and CSCC patients regarding DM. 40% of CSCC patients in this study had DM. An Insignificant statistical variance has been observed 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): Comparative analysis between group Ia (BCC cases) and Ib (CSCC cases) according to demographic and clinical data.

	Group Ia (BCC) (number = 15)		Group Ib (CSCC) (number = 15)		P
	No.	%	No.	%	
Gender					
Male	13	86.7	11	73.3	> 0.05
Female	2	13.3	4	26.7	
Age (years)					
Min. – Max.	37.0 – 75.0		25.0 – 80.0		> 0.05
Mean ± SD.	63.13 ± 10.15		63.60 ± 13.36		
Median (IQR)	63.0 (59.0 – 71.0)		66.0 (61.0 – 71.50)		
Sun exposure	13	86.7	12	80.0	> 0.05
Family history	0	0.0	0	0.0	–
Smoking	2	13.3	2	13.3	> 0.05
Comorbidities					
HTN	8	53.3	7	46.7	> 0.05
Virus C	2	13.3	4	26.7	> 0.05
DM	0	0.0	6	40.0	< 0.05*
Rt fibroadenoma resected	1	6.7	0	0.0	> 0.05
Number of lesions					
Single	13	86.7	9	60.0	> 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	> 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	> 0.05
Rubbery	2	13.3	2	13.3	
Firm	2	13.3	7	46.7	
TILs	2	13.3	0	0.0	> 0.05
LVI	0	0.0	1	6.7	> 0.05
Perineural invasion	0	0.0	0	0.0	–
Increase N/C					
No	10	66.7	10	66.7	> 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.

In this research 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 examined cases with regard 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 Ultrasound		
Free	12	80.0
Cervical Lymph nodes	1	6.67
Subcentimetric, upper and post cervical	1	6.67
Submental, submandibular, upper deep cervical	1	6.67

There was a significant statistical rise 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): Comparative analysis of the expression levels of studied long noncoding RNAs in skin tissue samples between groups Ia and IIa.

	Group Ia (number =15)	Group IIa (number =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.

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

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

	group Ib (number =15)	group IIb (number =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.

There was significant positive association 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						

*: Statistically significant.

A significant positive correlation has been found between expression levels of LINC00319 in skin tissue samples of CSCC patients and age. A significant positive association has been observed between expression levels of CASC15 in skin tissue samples of CSCC patients and T-staging. A significant positive association has been observed 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

*: Statistically significant.

DISCUSSION

Non-melanoma skin carcinoma is the most prevalent malignant tumor among the population, with a consistently rising prevalence attributed to an aging population and exposure to sun. The primary subtypes of non-melanoma skin cancer are cutaneous squamous cell carcinoma and basal cell carcinoma [(1)]. Non-coding RNAs (ncRNAs) have a crucial regulatory role in the progress of skin tumors. Long non-coding RNAs (LncRNAs) exhibit considerable versatility, functioning as scaffolds, decoys, or guides to alter essential signaling pathways, like the WNT/β-catenin pathway, and gene expression[(7)]. This research revealed the involvement of long noncoding ribonucleic acids CASC15, LINC00346, and LINC00319 in non-melanotic skin cancers, demonstrating their expression levels in both adjacent non-tumorous and tumor tissues utilizing RT-PCR analysis.

In this research, there was insignificant statistical variance 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 *et al.*** [(14)] reported that skin cancers usually progress in the elderly population, the mean age of cases was 71.4±11.05 years.

Similarly, **Ragi *et al.*** [(15)] stated in their clinical study a mean age of 71.8 years in skin cancer patients. The 1^{ry} reason for the increased prevalence of the illness in the elderly is their cumulative exposure to the sun over the years. The research found a statistically insignificant variance between BCC and CSCC cases concerning exposure to the sun and smoking, despite 86.7% of BCC cases and 80% of CSCC cases being chronically exposed to the sun and 13.3% of both groups being smokers. Increased UV exposure has been related to a heightened possibility of cutaneous squamous cell carcinoma and basal cell carcinoma.

The elevated relative possibility of keratinocyte carcinomas associated with increasing exposure to UV is more pronounced for CSCC (2.05; ninety-five percent Confidence interval, 1.54-2.73) compared to BCC (1.30; ninety-five percent CI, 1.18-1.43). The different effects of UV radiation on BCC and CSCC might partially explain the typically reduces BCC:CSCC ratios observed in southern areas [(12)].

Song *et al.* [(16)] indicated that individuals who have ever smoked had a slightly greater possibility of basal cell carcinoma (odds ratio (OR) 1.02, ninety-five percent confidence interval (CI) 1.00–1.04) and cutaneous squamous cell carcinoma (OR 1.08, ninety-five percent confidence interval 1.01–1.15) compared to those who have never smoked.

Leonardi-Bee *et al.* [(17)] documented a significant fifty percent elevation in the probability of cutaneous squamous cell carcinoma among ever smokers; nevertheless, no correlation was observed with basal cell carcinoma (OR, 0.95, ninety-five percent CI 0.82–1.09).

The recent United Kingdom Million Women research revealed that current smokers exhibited a higher frequency of squamous cell carcinoma in comparison with never smokers (RR 1.22, ninety-five percent CI 1.15–1.31), although the frequency of basal cell carcinoma was reduced (RR 0.80, ninety-five percent CI 0.78–0.82) [(18)].

Dusingize *et al.* [(19)] supported the outcomes of observational research associating smoking with reduced probability of BCC. However, no proof was found indicating that smoking is related to an increased risk of CSCC; in fact, their findings were more matched with a reduced risk, comparable to BCC.

In the present research, a significant statistical variance has been observed 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 findings are in accordance with **Dusingize *et al.*** [(19)].

Increasing data supports the hypothesis that diabetes mellitus, together with ultraviolet light, may contribute to the carcinogenesis of cutaneous squamous cell carcinoma. Cases with diabetes mellitus are more susceptible to infections and commonly present with chronic ulcers in the lower limbs. Patients of cutaneous squamous cell carcinoma rising in long-standing ulcers on the feet of diabetic cases have been documented as a complication of diabetes mellitus [(20,21)]. Marjolin's ulcer is an uncommon yet aggressive kind of cutaneous squamous cell carcinoma that develops in chronic ulcers resulting from diabetic neuropathy, vascular insufficiency, pressure, hemoglobinopathy, or inside scar tissue [(8)].

Also, **Larijani *et al.*** [(22)] described in their research that in chronic, nonhealing diabetic foot ulcers with the presence of old age and sun exposure risk factors, diagnosis of SCC should be taken into account and excluded. We found in our research that most of CSCC cases 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 cases (80%) in this research had no involved lymph nodes (free), which is supported by **Dusingize *et al.*** [(19)] who reported that most of CSCC tends to have a promising prognosis with elevated rates of local control and rates of distant metastasis as low as two to three percent. Generally, between five and twenty percent of cases have nodal metastases from cutaneous squamous cell carcinoma.

This research illustrated a statistically significant elevation in the expression levels of CASC15 long noncoding RNA in skin tissue samples from BCC cases compared to controls. These findings agree with previous study[(23)], which indicated that CASC15 is increased in BCC. Our findings have been supported by **Sheng and Wei** [(7)], who indicated that CASC15 promotes proliferation of cell and suppress apoptosis through activating the WNT/β-catenin signaling pathway, as well as by **Noubissi *et al.*** [(24)] who noted that

this pathway is included in the progression of basal cell carcinoma and is essential for the proliferation and patterning of both embryonic and adult tissues.

The WNT signaling pathway enhances the transcriptional output of Hh signaling, with Hh activation serving as a crucial factor in basal cell carcinoma progression. WNT signaling promotes the expression of insulin-like growth factor 2 mRNA-binding protein or RNA-coding region determinant binding protein (CRD-BP) 1 (IGF2BP1), which subsequently binds to and stabilizes GLI1 mRNA. This process results in the upregulation of transcriptional activity and GLI1 expression, leading to persistent activation of Hh signaling and unregulated canonical cell proliferation [24].

Although recent research [25] has recognized CASC15 as an oncogene in other cancers, including tongue squamous cell carcinoma, oral squamous cell carcinoma, cervical cancer, and melanoma, its function in cutaneous squamous cell carcinoma is still unclear. To examine the biological function of CASC15 in cutaneous squamous cell carcinoma, we conducted qRT-PCR research to assess its expression in 15 paired cutaneous squamous cell carcinoma tissues and adjacent noncancerous tissues, revealing that CASC15 was markedly increased in cutaneous squamous cell carcinoma tissues relative to matched nontumorous skin tissues. Our outcomes may be supported by **Sheng and Wei** [7], which indicated that CASC15 promotes proliferation of cell and inhibits apoptosis through the activation of the WNT/β-catenin signaling pathway, and by **Takada** [26], who stated that WNT/β-catenin signaling is crucial for the initiation and progression of HPV-driven CSCC, as well as for preserving the cancer stem cell niche. HPV-induced cutaneous squamous cell carcinoma is the predominant tumor among immunosuppressed organ transplant recipients (OTR), with as many as fifty percent of these cases developing CSCC within ten years post-transplantation [27].

Recent findings indicate that LINC00346 and LINC00319 lncRNAs are increased in CSCC [8,9], although their function in BCC remains unknown. LINC00346 enhances the expression of MMP-3, MMP-13, MMP-10, and MMP-1 [8]. LINC00319 enhances the expression of MMP-9, MMP-2, and markers associated with epithelial–mesenchymal transition, specifically vimentin and E-cadherin [20]. MMPs have a role in the formation of BCC and growth of tumors. The secretion of MMP-1 and MMP-2 by fibroblasts promotes tumor development. MMP-13, released by fibroblasts and neoplastic cells, facilitates tumor angiogenesis. MMP-9 released by inflammatory cells stimulates BCC cells to produce VEGF, hence facilitating angiogenesis. [28]. LINC00346 controls the activity and expression of STAT3, therefore enhancing the expression of matrix metalloproteinases MMP-3, MMP-1, MMP-13, and MMP-10 [8]. STAT3 signaling pathway is involved in BCC carcinogenesis, throughout the progress of ultraviolet (UV) B skin cancers, STAT3 has a vital role in both proliferation and survival of keratinocytes [29].

We also found in our research a significant statistical rise of the expression levels of LINC00346 long noncoding RNA in skin tissue samples of CSCC patients compared with their controls. These outcomes are in line with **Piipponen *et al.*** [30] who reported in their research that there is a significant elevation of PRECSIT has been found in CSCC cell lines than normal skin tissue samples. Also, they identified PRECSIT (LINC00346) as one of the most elevated lncRNAs in cutaneous squamous cell carcinoma cells with very low expression in normal human epidermal keratinocytes (NHEKs) based on RNA-seq. **Zhou *et al.*** [31] have found that Jak-STAT signaling is one of the most possible targets for PRECSIT. Furthermore, a significant down-regulation of STAT3 mRNA and protein concentrations following knockdown of PRECSIT has been seen, leading to a potent decrease in activated STAT3 levels, suggesting that PRECSIT's action is mediated through STAT3 signaling in CSCC.

In our research we observed a significant statistical rise 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 **Yuan *et al.*** [9] who reported that LINC00319 expression has been recognized to be raised in CSCC with researches proposing its association with bad prognosis is because of its ability to promote cell invasion and proliferation. **Li *et al.*** [32] conducted qRT-PCR analysis to assess LINC00319 expression levels in sixty paired cutaneous squamous cell carcinoma tissues and adjacent noncancerous skin samples, revealing that LINC00319 was considerably elevated in cutaneous squamous cell carcinoma tissues relative to matched non-tumorous tissues. Furthermore, **Piipponen *et al.*** [30] indicated that LINC00319 is increased in CSCC, promoting cell growth, invasion, and migration though suppressing apoptosis through the upregulation of cyclin-dependent kinase 3 through miR-1207-5p decoy. LINC00319 may competitively associate with miR-1207-5p in CSCC cells.

Furthermore, CDK3, a member of the cyclin-dependent kinases family, has a complex relation to the G1/S and G0/G1 transitions of the cell cycle, was recognized as a direct target of miR-1207-5p in cutaneous squamous cell carcinoma cells. LINC00319 has been discovered to control CDK3 expression through sponging miR-1207-5p in CSCC cells [31].

We observed insignificant statistical variance between BCC patients and CSCC cases 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 detected a significant positive relation between expression levels of the three studied LNCRNAs in cancerous skin tissue samples of BCC and CSCC patients.

In this research we found a significant positive association 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 additionally found a significant positive association 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 *et al.*** [30] found that the percent of PRECSIT-positive cells was significantly greater in invasive cutaneous squamous cell carcinoma compared to in noninvasive cutaneous squamous cell carcinoma precursor lesions as AK, and higher in AK than normal skin. In this research we found a significant positive association between expression levels of LINC00319 in skin tissue samples of group CSCC patients and age. In contrast **Li *et al.*** [32] found a non-significant positive association 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 research was carried out on sixty paired tumorous tissues and matched nontumorous tissues have been gathered from cutaneous squamous cell carcinoma cases. 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 *et al.*** [32] reported that elevated expression of LINC00319 was related to larger size of cancer, lymphovascular invasion, and advanced TNM stage and suggests a possible association with poor prognosis and illustrates CSCC development.

CONCLUSION

The three studied LncRNAs are upregulated in BCC and CSCC skin tissue samples. There is significant positive association between expression levels of the three studied LNCRNAs in skin tissue samples of BCC and CSCC cases. 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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