RESEARCH ARTICLE
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ADVANCING ORAL CANCER DIAGNOSIS VIA TGFB SIGNALING PATHWAY

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Abstract
Background: The risk to a person's life posed by oral cancer can be significantly reduced if it is identified and confirmed early in the course of the illness. As a regulator, transforming growth factor-β (TGFβ) is essential to the control of numerous biological processes. Oral cancer patients' salivary TGFβ levels increase. It might therefore be applied as a diagnostic biomarker. The objective of this research was to create a reporter system in yeast cells that can identify TGFβ, serving as a non-invasive means of screening for oral cancer. **Methodology:** Transformation of yeast cells was verified by restriction digestion after transforming growth factor beta receptor II (TGFβRII) and collagen type II alpha chain I (col2a1) (intronic region) carrying vector were added. Gel electrophoresis analysis was used to express, optimise, and establish the recombinant proteins. Additionally, saliva from patients with oral cancer was combined with recombinant yeast cells, and the results were evaluated using green fluorescence protein fluorescence microscopy and the enzyme-linked immunosorbent assay (ELISA). **Results:** It was discovered that there was a substantial difference in TGFβ expression (P value < 0.05) between yeast cells that had undergone transformation and those that had not. Oral cancer patients' saliva samples were tested for TGFβ using ELISA, and samples of yeast treated with saliva were tested for TGFβ using solid-phase sandwich ELISA. The yeast samples containing TGFβRII and Col2a1 expression vectors showed the highest fluorescence. **Conclusion:** In this work, a green fluorescent protein reporter system based on yeast was developed for molecular detection of early oral cancer via saliva. ELISA and fluorescence microscopy were used to optimise and detect TGFβRII protein expression in S. cerevisiae. Oral cancer can be diagnosed non-invasively with this co-transformed method.

Keywords: TGFβRII, ELISA, Transformation, TGFβ, Col2a1 and Oral cancer

INTRODUCTION

One of the most frequent malignancies in both industrialised and developing nations is oral cancer. Transforming growth factor expression (TGFβ) is present in every cell in the human body and is essential for numerous cellular developmental processes as well as homeostasis (Pylayeva-Gupta, 2011; Ryan, Poddar, & Cate, 2016). These growth factors are crucial for controlling the multitude of biological events that occur during the growth and development of the embryo (Tan Timur et al.,

2019). Numerous specialists' experiments and a review of the literature revealed connections between the receptors involved in TGFβ signalling, such as the kinase activity of TGFβRI and TGFβRII. In vitro and in vivo analyses of experiments, however, suggested a connection between the TGFβ signalling system and the promotion or onset of cancer (Nickel, Ten Dijke, & Mueller, 2018).
Encoding a pro-alpha-1 (II) series of type II collagen, which is primarily found in cartilage and the vitreous humour of the eye, is directly linked to the Col2a1 gene, which is specifically marked to have 54 exons and approximately 31.5 kb of genomic DNA located at 12q13.11, ~ 47 Mb from p-telomere. As a result, it has been suggested that abnormalities in the Col2a1 gene may be the associated cause of a number of diseases, including type II collagen disorders (Cheah, Stoker, Griffin, Grosveld, & Solomon, 1985; Khurshid et al., 2018; Lafont, Poujade, Pasdeloup, Neyret, & Mallein-Gerin, 2016). Green fluorescent proteins and the luciferase test can be used to quantify the expression of the linked gene, and the transcriptional reporter system Col2a1 is intended to monitor this expression (Kan et al., 2009; Peng et al., 2008).
It has been demonstrated that yeast S. cerevisiae responds to intricate signalling networks with remarkable efficiency. Many disorders, including cancer and metabolic syndromes, have been studied and analysed by the manipulation of yeast transformation with different genes (De la Vega et al., 2019). In order to detect non-invasive oral cancer, this study used a single yeast cell to co-transform the genes TGFβRII and Col2a1, demonstrating the effective transformation of a single gene in yeast (Brückner, Polge, Lentze, Auerbach, & Schlattner, 2009).
By examining the detectable tumour DNA in biological fluids such blood, serum, and saliva from individuals with head and neck cancer, oral cancer was discovered. On the other hand, a less intrusive and uncomfortable technique for finding the biomarker in oral cancer was saliva sample analysis (Fields & Song, 1989). Because saliva samples from oral cancer patients don't worsen, we have used them for our studies. Since human saliva is more readily available, it has been shown to be a more useful sample. It was also an accessible way to perform research in big demographic groups (Schmidt, Kulasinghe, Perry, Nelson, & Punyadeera, 2016).
This project investigated a yeast based TGFβ Signaling expression system which is completely non-invasive, for early detection and rapid diagnosis of oral cancer patients through saliva.

Materials and methods

Study design and sample collection:

A retro-prospective nature cohort study has been carried out by us. We primarily used two primary steps in our experimental study. The initial stage was co-transforming vectors containing TGFβRII and GFP genes in Saccharomyces cerevisiae and examining the kinetics of TGFβR protein expression within a yeast cell. Improve the reporter system test further to identify TGFβ cytokine. The second phase involved gathering data mostly from 60 patients in different tertiary and palliative care institutions in Lahore, Pakistan, who had been provisionally diagnosed with oral cancer. Based on our study's statistical setup, of the 60 patients who were initially diagnosed with oral cancer, 20 were classified as normal, and 40 were given a definitive diagnosis. The project was approved by the ethical committee of the Institute of molecular biology, and biotechnology (IMBB/UOL/20/137), and research experiments have been conducted according to international and institutional guidelines.
The samples that were obtained from each cohort were subjected to a rigorous evaluation process using six parameters, the majority of which are components of the solid phase sandwich ELISA. The review report was provided by a medically certified pathologist to help with the diagnosis of separating the healthy from the cancerous portion of the slide specimen. The patient who was diagnosed has several anatomical regions of the head and neck affected by oral cancer. We developed our inclusion criteria in a really unique way. We have only included people in our study who voluntarily agreed to take part in it and who formally granted us permission to do so. Individuals who have never had cancer previously, are at least thirty years old, and have never had therapeutic or palliative care.

Furthermore, only those who have never had diabetes, heart problems, or taken medicine for any of these disorders in the past. Everyone who didn't fit our requirements for inclusion was taken out of the research.

Strains, Vectors and Media

We bought two vectors with a eukaryotic promoter system from ADD GENE. The TGFβRII gene's DNA sequence was present in one vector, while the green fluorescence gene with the Col2a1 enhancer's upstream region was present in the other (Figure 1a & b).

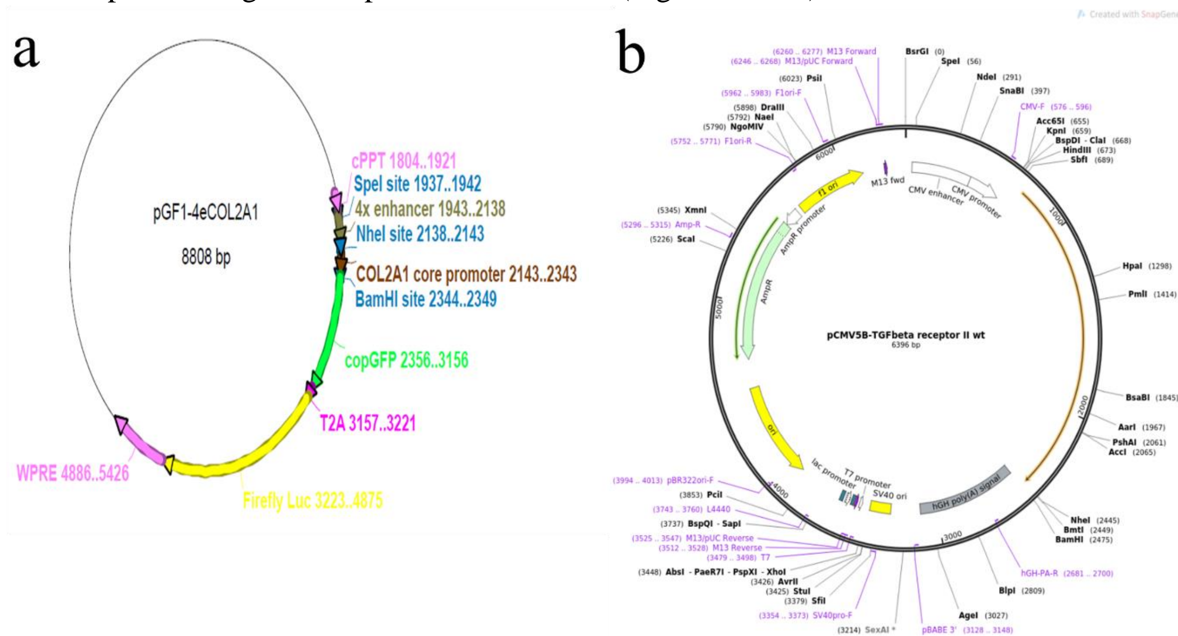

Figure 1: a) pGF1 map for cloning of Col2a1 (restriction enzymes sites are *Spe*I and *Bam*HI, Col2a1 promoter along with four repeated enhancer region are shown). **b)** pCMV5B map for cloning of TGFβ receptor 2, the restriction enzyme sites are BamHI and KpnI, and the total plasmid size is 6396.

Vectors for plasmids were propagated using E. Coli DH5 alpha cells. The University of Lahore, Pakistan's Center for Research in Molecular Medicine, Institute of Molecular Biology and Biotechnology (CRiMM/IMBB) had E. Coli DH5 alpha cells on hand. S. cerevisiae was the yeast strain employed for eukaryotic expression. LB agar was utilized to support the growth of the E. Coli DH5 alpha bacterial strain, whereas YPD medium was utilized to keep S. cerevisiae alive. Using normal procedures, the vector-containing cells were cultured on agar plates treated with ampicillin (Ovchinnikov et al., 2014). TGFβRII and Col2a1 plasmids were extracted, and 1% agarose gel electrophoresis was used to confirm the findings.

Transformation of TGFβRII and Col2a1 Containing Vectors

The lab was used to produce yeast cells for the transformation. Little modifications were made to the LiAc/SS Conveyor DNA/PEG transformation procedure as described by Gietz and Schiestl (2007) in order to transform S. Cerevisiae competent cells (SANTAMARiA, Gil, Mesas, & Martín, 1984). The TGFβRII plasmid-transformed colonies were identified by their ability to thrive on media treated with ampicillin. Col2a1 plasmid was used to convert yeast that expressed TGFβRII. The altered colonies were isolated by selection on a medium supplemented with ampicillin.

Confirmation of Transformation

Single-enzyme digestion was carried out to verify the transformation of yeast. For one hour, the isolated plasmid was treated at 37°C with 1µl of the restriction enzyme BamHI. Under UV light, the digested DNA was visible on a gel electrophoresis. Colony PCR was used to verify TGFβRII and Col2a1 plasmid transformation. A 20µl final volume was used for the polymerase chain reaction

(PCR), which was produced with 1X PCR buffer (5µl), MgCl2 (3µl), dNTPs (0.5µl) of each primer, Taq polymerase (0.5µl), and 10µl of the boiling transformed colony supernatant.

PCR thermocycler settings were as follows:
Plan was created, which included five minutes of initial DNA denaturation at 95 °C and thirty cycles of denaturation for one minute at 94 °C, annealing at 62 °C for Col2a1 and 60 °C for "TGFβRII specific primers" for forty seconds, and an extension for one minute at 72 °C for DNA elongation. Finally, each PCR reaction was allowed to amplify for five minutes at 720 C. Under UV illumination, 1% gel electrophoresis was used to display the findings of each PCR product.

Gene Expression Analysis of TGFβRII and GFP
Pre-stained protein ladder (LC5925, Invitrogen) was used in a 12.5% SDS-PAGE run to determine the expression level of TGFβRII protein. Samples of the yeast culture that had undergone transformation were obtained every two hours, processed, and run on a 12.5% SDS-PAGE.

Gathering of Serum and Saliva Samples
In addition, 5 milliliters of entire saliva were taken from both oral cancer patients and controls. The blood samples were centrifuged at 1500 rpm for 15 minutes at room temperature, and the serum was recovered and kept at -80°C until analysis.

Evaluation of Cytokines Level
Patients and healthy individuals provided saliva and serum samples for the sandwich ELISA (enzyme linked immunosorbent test), which was used to assess six parameters. These included interleukin-6 (IL-6), interleukin-1 (IL-1), P53, vascular endothelial growth factor (VEGF), tumor necrosis factor alpha (TNF-α), and TGFβ. Additionally, TGFβRII activity was assessed via ELISA, which took advantage of the antigen-antibody interaction between TGFβRII antibody and the receptor protein TGFβRII from yeast lysate. The ELISA protocol, as outlined in Wajid et al. (2016) (Wajid et al., 2015), was adhered to (Gietz, 2014).
The anti-P53 (catalog no. sc-393031), anti-TNFα (catalog no. sc-515765), anti-VEGF (catalog no. sc-57496), anti-TGFβ (catalog no. 65378), anti-IL-6 (catalog no. ab9324), and anti-IL-1 (catalog no. ab2105) antibodies were acquired from Santa Cruz Biotechnology USA. The blocking solution and the cultured plate were incubated for 24 hours at 4°C after the coating buffer (100µl) was used to dilute the solution and the plate was repeatedly rinsed with TBS (tris buffered saline) solution. The wells were then rewashed with Tris-buffered saline (TBS) and left overnight to be treated with a secondary antibody conjugated with horseradish peroxidase (HRP) (Santa Cruz Biotechnology, USA). To get rid of a secondary antibody, the plate was repeatedly cleaned. To find a secondary antibody, 3,3',5,5'-Tetramethylbenzidine (TMB) (Invitrogen Inc., USA) chromogenic solution was used. Sulfuric acid was treated in order to halt the chromogenic process. Together with 650 nm as a reference value, absorbance was measured at 450 nm.

Flouresence Microscopy
The procedure for fluorescence microscopy was as previously described (Kaishima, Ishii, Matsuno, Fukuda, & Kondo, 2016; Wajid et al., 2015). Harvested cells are reorganized after being rinsed in distilled water. A BIOREVO BZ-9000 fluorescent microscope (Keynes, Osaka, Japan) with a 40X objective lens was used to study the cell suspension. An image of green fluorescence was captured using a 470/40 band-pass filter for excitation and an emission-side 535/50 band-pass filter. 20µm is the scale bar. It was exposed for 1/15 seconds. Normal yeast cells were found in the collected cells.

Statistical Analysis
Statistical analysis was performed on the complete data from our experimental cohort study, which included both cancer and normal individuals. To determine the normality distribution and reliability

of the data, descriptive statistical analysis and t-test analysis of the six parameters of TGFβ, VEGF, TNF-α, P53, IL-6, and IL-1 were carried out. Each and every value was given as mean ±SEM. When necessary, the experiment was conducted in triplicate.

Results

Transformation of TGFβRII and Col2a1

By screening the pCMV5B-TGFβ plasmid on gel, which subsequently indicates the existence of big bands under gel electrophoresis, the presence of plasmid cells was verified. Since the plasmid contained an ampicillin resistance gene, the colonies harboring TGFβRII and Col2a1 were later screened on media by growing colonies coated with ampicillin. In comparison to the TGFβ plasmid band on pCMV5B, the latter band was slightly heavier.

Confirmation of Transformation

Enzyme digestion was performed on the isolated plasmids to verify the existence of both the pCMV5B-TGFβ plasmid and the pTGF1-Col2a1 plasmid. The plasmids were treated with the common enzyme BamH1 for three hours after it was chosen for digestion. The single cut made by this enzyme on both plasmids produced the two bands on the agarose gel that are depicted in Figure 2a.

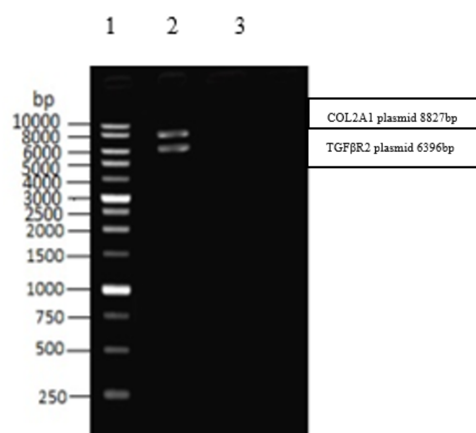

Figure 2a: Lane 1: The result of the two introduced plasmids that were separated from the transformed yeast cell after restriction digestion. Lane 2: According to the lane gene ladder, the second band is located at 6396 bp and the size of the top first band is 8827 bp. Lane 3: Negative control.

Through the use of end-point PCR, co-transformation was further confirmed. Figure 2b shows the band of 1800 bp that was obtained from gel electrophoresis after the TGFβRII receptor gene was amplified. Similarly, PCR was performed on altered yeast cells. To validate the intronic region of the Col2a1 enhancer, 407 bp band was visible on the gel electrophoresis, as seen in figure 2c.

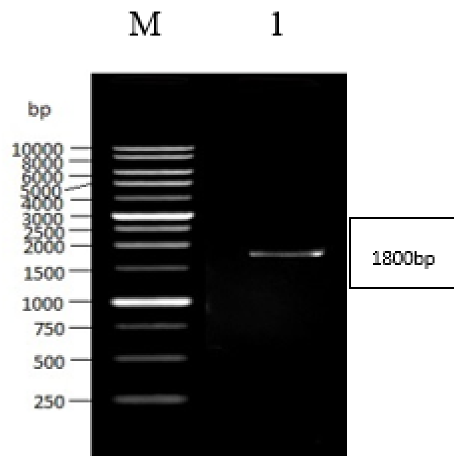

Figure 2(b): The gene ladder was conducted in lane M, while the band size in lane 1 is 1800 bp. The size of the insert has verified that TGFβRII transformation is present in yeast cells.

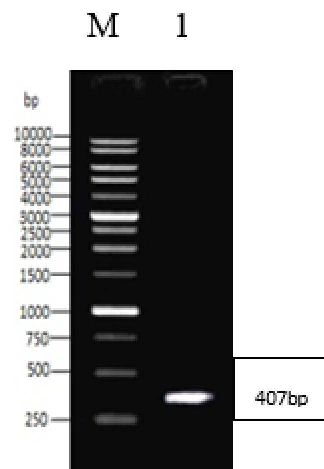

Figure 2(c): The gene ladder was conducted in lane 1, but the band size in lane M is 407 bp. The presence of Col2a1 transformation in yeast cells has been established by the insert size.

The co-transformation of the TGFβRII gene and the Col2a1 gene was validated by the PCR and enzyme digestion data mentioned above.

Gene Expression Analysis of TGFβRII and GFP

After increasing the incubation period, proteins were extracted (lane 1 to lane 10). Figure 3 illustrates the increase in recombinant protein production at the 120-hour incubation period.

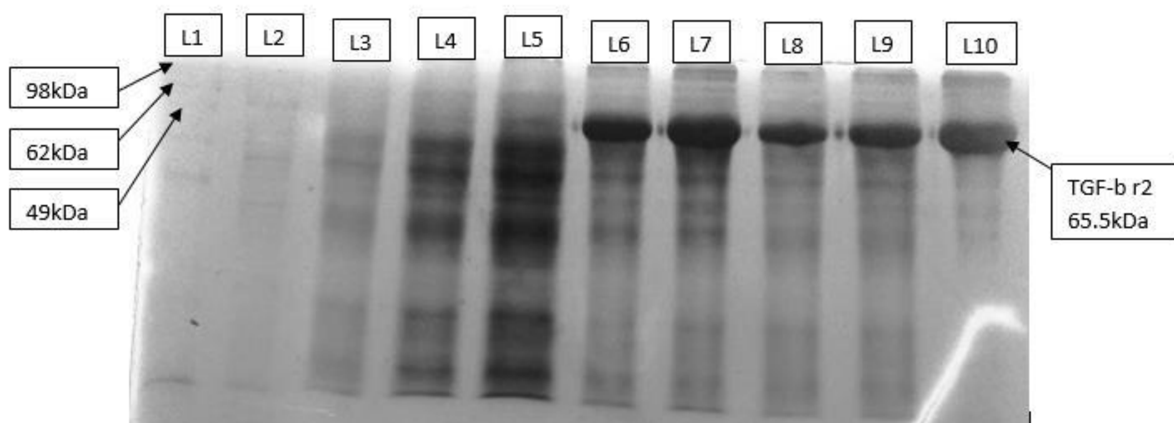

Figure 3: TGFβRII protein on a 12.5% SDS PAGE. Protein ladder pre-stained in Lane 1. Lane 2: 15µL of untransformed yeast cells Lane 3: After 24 hours, TGFβRII in co-transformed yeast cells Lane 4: 48 hours after co-transformation, TGFβRII in 15µL of yeast cells. Lane 5: 72 hours later, TGFβRII in co-transformed yeast cells Lane 6 displays the TGFβRII in co-transformed yeast cells at 96 hours (15µL), while Lane 7 displays the same information at 120 hours. Lane 8: At 120 hours, 15µL of co-transformed yeast cells expressing TGFβRII. Lane 9: 120 hours later, TGFβRII in co-transformed yeast cells. Lane 10: 120 hours later, TGFβRII in co-transformed yeast cells.

Fluorescence Microscopy

Following treatment with saliva from patients with oral cancer, TGFβ was employed as a substrate to detect activation of Col2GF reporter activity in transformed yeast cells by fluorescence microscopy. Figure 4 shows the optimal incubation period of 96 hours that produced the desired findings.

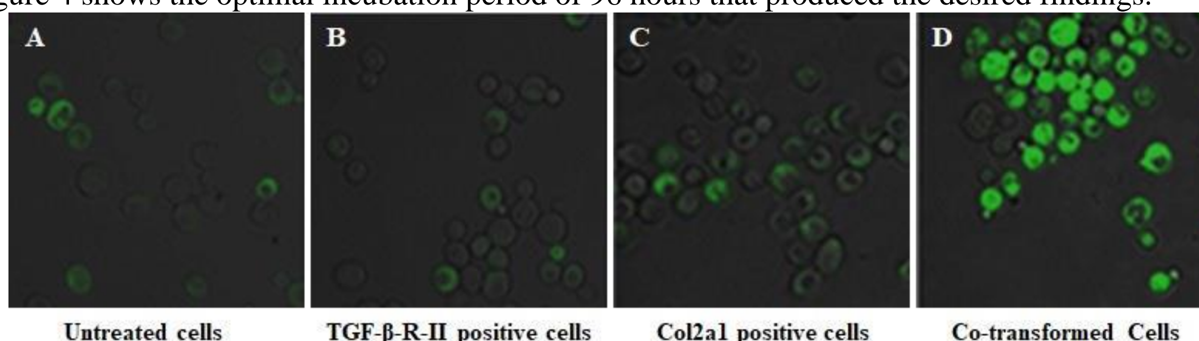

Figure 4: Saliva from patients with oral cancer showed plasmids expressing Col2GF. (a) Expression of yeast cells left untreated and not exposed to patient saliva. (b) The TGFβ-receptor II plasmid's reaction with the patient's saliva. (c) The Col2a1 plasmid's reaction with the patient's saliva. (d) Saliva from oral cancer patients treated with co-transformed plasmids had the highest expression of fluorescence. Using a fluorescence microscope with a 40× objective lens, the pictures were taken. 20 µm is the scale bar. It was exposed for 1/15 seconds.

Cytokines and Growth factor analysis

TGFβ, VEGF, TNF-α, P53, IL-6, and IL-1 were among the cytokines that were expressed more in the saliva and serum of patients compared to controls ($p<0.005$). Normal samples had a size of 20, while diseased samples had 40. Yeast cells undergoing co-transformation had a large P value. ($p<0.05$), however there was no significant difference between yeast cells cloned with the TGFβRII and Col2a1 plasmids separately ($p=0.216$). Using an Elisa plate reader set to 450 nm, samples were measured.

Saliva of Oral Cancer Patients' Levels of Growth Factor and Cytokines

Sandwich ELISA was utilized to measure growth factors (TGFβ, VEGF, P53, TNF-α, IL-6, and IL-1) in the saliva of patients with cancer and those without it in different ways. Saliva from oral cancer patients had considerably lower levels of p53 (0.074 ± 0.0039) than saliva from normal patients (0.16 ± 0.0072). On the other hand, compared to normal patients (0.17 ± 0.011), oral cancer patients had significantly higher levels of TGFβ (1.6 ± 0.030), VEGF (1.32 ± 0.0835), and TNF-α (1.6 ± 0.043) than normal patients (0.27 ± 0.020).

Saliva from patients with oral cancer had a greater amount of IL-1 (1.8 ± 0.037) compared to normal patient saliva (0.29 ± 0.016). Similarly, saliva from oral cancer patients had greater levels of IL-6 (1.5 ± 0.060) than saliva from normal patients (0.14 ± 0.010). Figure 5 illustrates the statistical significance of the difference between them.

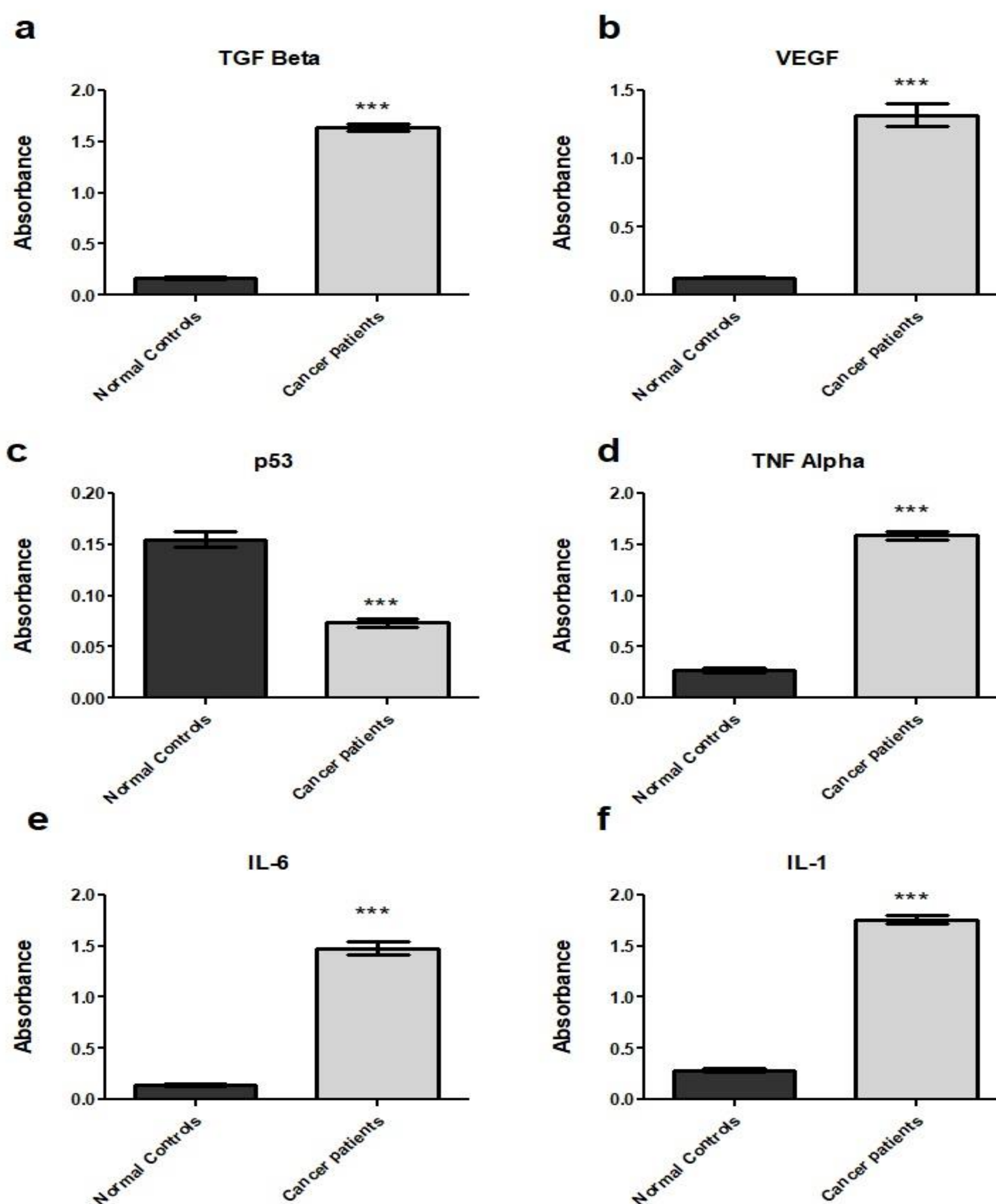

Figure 5: In both normal and oral cancer patients, the expression of saliva cancer markers is shown by a bar graph. (a) Displays increased TGFβ expression in saliva. (b) Exhibits elevated VEGF expression in saliva. (c) Indicates a lower P53 level in the saliva. (d) Indicates an increase in salivary TNF-α levels. (e) Exhibits increased IL-6 expression in saliva. (f) Indicates that salivary IL-1 is overexpressed. To graphically represent the frequency distribution, a bar graph is created. The relative distribution of each category of cancer indicators is displayed by the height of the bar. Values were presented as ±SEM and the T-test (nonparametric) was used. There is a considerable difference between cancer patients and normal individuals, with $p<0.05$ considered significant. There are 60 patients in the sample (20 normal and 40 cancer patients).

Level of Growth Factor and Cytokines in Serum of the Oral Cancer Patients

The growth factors (TGFβ, VEGF, P53, TNF-α, IL-6, and IL-1) in the serum of both malignant and normal individuals were assessed differently using sandwich ELISA. Patients with oral cancer had serum levels of p53 that were considerably lower (0.051 ± 0.0028) than those of normal patients (0.089 ± 0.0020). In contrast, oral cancer patients' serum levels of TGFβ (1.8 ± 0.053) were significantly higher than normal patients' (0.13 ± 0.0098) and VEGF (2.33 ± 0.0401) were significantly higher in oral cancer patients' serum than in normal patients' (0.0793 ± 0.00345). In

addition, the serum level of TNF-α in patients with oral cancer was substantially higher (2.4 ± 0.061) than in normal patients (0.071 ± 0.0043). Likewise, patients with oral cancer had serum levels of IL-1 that were greater (2.5 ± 0.054) than those of normal patients (0.073 ± 0.0038). In addition, patients with oral cancer had higher serum levels of IL-6 (2.4 ± 0.056) than did normal patients (0.099 ± 0.0051). Figure 6 illustrates the statistical significance of the difference between them.

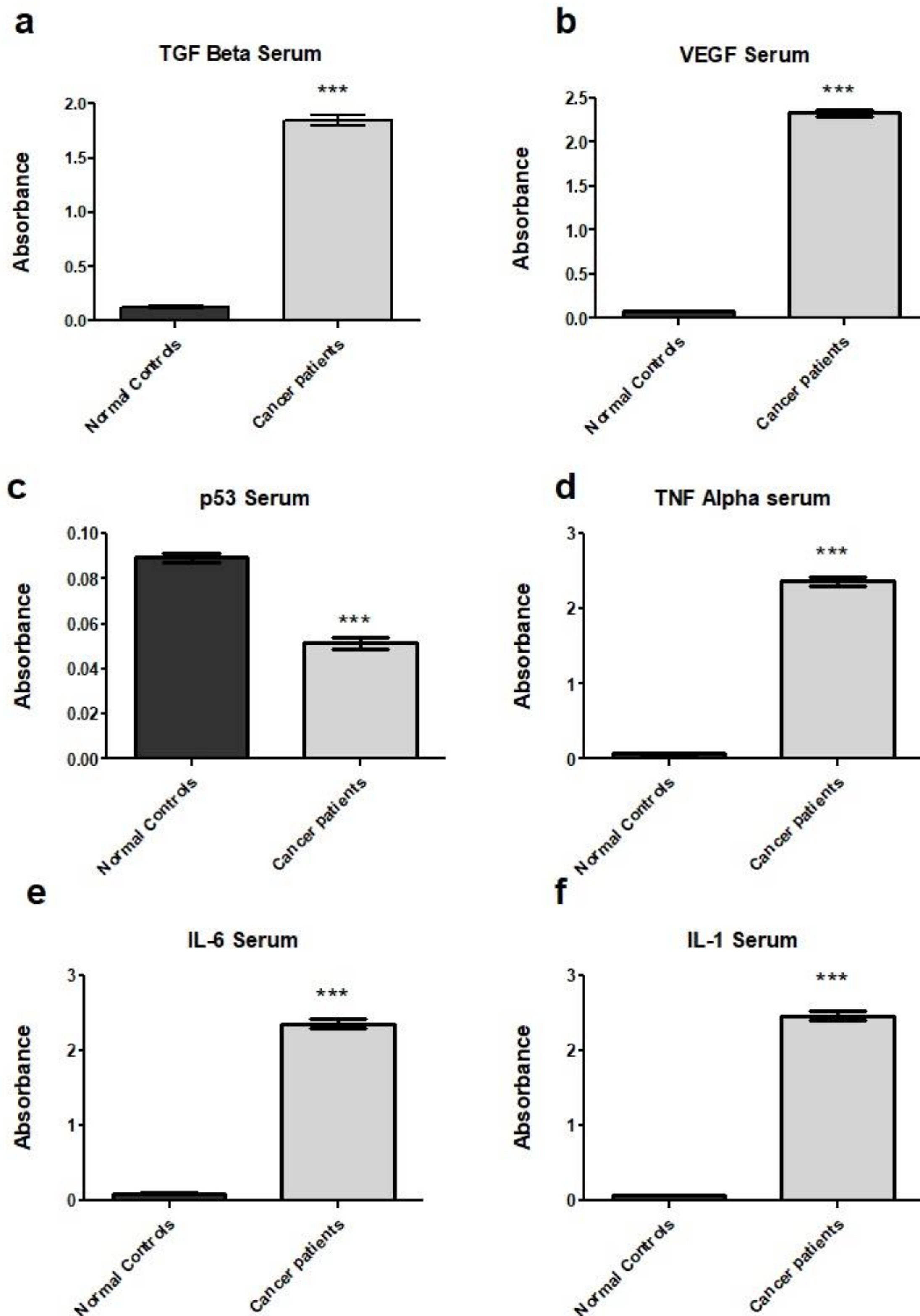

Figure 6: The expression of serum cancer markers in patients with oral cancer and healthy individuals is shown as a bar graph. (a) Displays elevated TGFβ expression in serum. (b) Exhibits elevated VEGF expression in serum. (c) Indicates a drop in serum P53 levels. (d) Exhibits elevated

serum TNF-α expression. (e) Indicates that serum IL-6 is regulated. (f) Exhibits elevated IL-1 expression in serum. To visualize the distribution, a bar graph is created. The relative distribution of each category of cancer indicators is displayed by the height of the bar. The values were presented as mean ±SEM and the T-test was used. There is a considerable difference between cancer patients and normal individuals, with $p<0.05$ considered significant.
Sixty patients (20 normal and 40 cancer patients) make up the sample size.

TGFβRII signaling activity

Through the use of TGFβ-receptor II antibody and the antigen-antibody interaction of the receptor protein TGFβRII from yeast lysate, TGFβ-receptor II receptor activity was assessed using the enzyme linked immunosorbent test (ELISA). Figure 7 depicts the groups of yeast cells that were transformed and untransformed after being exposed to the saliva of individuals with oral cancer.

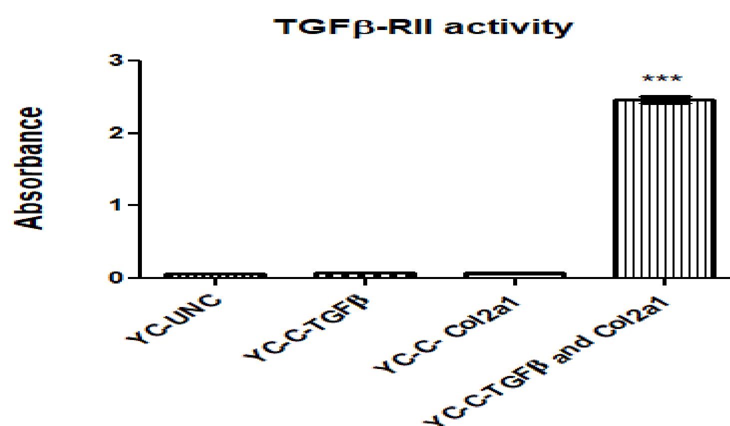

Figure 7: Following a response with the saliva of patients with oral cancer, the bar graph displays the TGFβ Lysate expressions according to plasmids. To visualize the distribution, a bar graph is created. The bar's height indicates a related distribution of TGFβ yeast lysate expression in each group. The final bar illustrates the increased quantity of yeast cells containing co-transformation 2 plasmids in comparison to uncloned yeast cells, cloned yeast cells with TGFβ plasmid, and cloned yeast cells with Col2a1 plasmid separately. Values were represented as mean ±SEM using a one-way ANOVA with Bonferroni's posttest; $p<0.05$ was considered significant.

Discussion

The sixth most common cancer with the highest death rate is oral cancer. More than 90% of oral malignancies are oral squamous cell carcinomas. Because saliva collection and sampling methods are simple and noninvasive, there is hope for the early identification of cancer through the use of saliva. It has already been determined that more than 100 salivary biomarkers, including cytokines (IL6, IL-1, TNF alpha, and P53), exist (Pylayeva-Gupta, 2011).
The yeast used in this investigation was transformed with two circular plasmids: pGF1- 4eCol2a1 (intronic region) and pCMV5B-TGFβ receptor II (TGFβ receptor II gene). The expression of the former Col2a1 was triggered by the latter protein, TGFβ receptor II (Ryan et al., 2016).
TGFβ has been found to bind to the sort II receptor, a transmembrane protein with a cytoplasmic serine/threonine kinase domain, with an excessive amount of affinity (Ishii et al., 2014; Meng, Nikolic-Paterson, & Lan, 2016; Tan Timur et al., 2019). It was observed that the early gene response was inhibited by type II receptor-dependent TGFβ binding protein association, kinase activity, and type I receptor (Vander Ark, Cao, & Li, 2018). The TGFβRII constructed plasmid is sufficient for mediating BUB1 (budding uninhibited by benzimidazoles 1) phosphorylation of the SMAD2-TGFβR structural fragmentation, thus regulating the TGFβ signature event, even though the TGFβ signalling pathway is efficient when bound with TGFβRI and TGFβRII receptors for its activation (Kwak, Kim, Kim, & Choi, 2008).
Six criteria for detecting oral cancer in serum and saliva samples were covered in this investigation. TGFβ, VEGF, TNF alpha, P53, IL-6, and IL-1 are these parameters. It is thought that alterations in

the oral epithelium initially cause dysplasia of the oral cells, which in turn causes cell cancer. This procedure may involve multiple components. The crucial cytokine Transforming Growth Factor-beta (TGFβ) triggers the epithelial mesenchymal transition (EMT). TGFβ is a member of the superfamily of proteins, which also includes growth factors, activins, and bone morphogenetic proteins. TGFβ is a multifaceted cytokine that is crucial for the growth, development, and migration of cancer cells (Liu et al., 1999). According to our research, TGFβ levels in patients with oral cancer are higher than in normal patients. Xin-Pang et al.'s 2018 study also supported this finding, indicating that TGFβ has conflicting effects on cancer and functions as a tumour suppressor in the early stages of carcinogenesis.

Transforming Growth Factor Receptor Type II, or TGFβRII, is a ruse in the yeast two-hybrid system because TGFβ's effects are mediated by a heteromeric complex of type I and type II threonine kinase receptors. Because of the downstream substrate, TGFβRII binds to TGFβ and phosphorylates constitutively, whereas TGFβRII cannot bind its ligand without TGFβRII (Ghazi, Saghravanian, Shakeri, & Jamali, 2021). Since yeast has a signal transduction mechanism that is similar to a signalling route mediated by human TGFβ, the cloned subtype of TGFβRII's 25-amino acid insertion sequence is acquired in relation to the type-II receptor's receptor sequence (Liu et al., 1999).

The expression of Col2a1 can be driven by TGFβ superfamily signalling in culture conditions that mimic physiological signals. It raises protein secretion, TGFβ bioactivity, and TGFβ2 mRNA expression. Col2a1 gene expression was upregulated upon TGFβ2 isoform knockdown under conditions of increased TGFβ bioactivity (Ryan et al., 2016). TGFβ in chondrocytes is implicated in the transcription of collagen type II. After TGFβ ligand binds to TGFβRII (TGFβ type II receptor), ALK5 (type I receptor activin-like kinase 5) is recruited, and intracellular effector molecules SMAD-2 and -3 are phosphorylated to control transcriptional activity (Nikawa, 1994).

Fluorescence microscopy is utilised to identify the activation of Col2GF reporter activity in yeast cells that have been transformed, using TGFβ as a substrate. To maximise the fluorescence signal, different dilutions of TGFβ substrates (24, 48, 96, and 120 hours) have been applied to the altered yeast cell.

Early cancer stages are associated with increased TGFβ expression in saliva, therefore we evaluated the amount of this cytokine in cancer patients' saliva and let it interact with recombinant yeast cells. TGFβ is indicated by the presence of a GFP signal. TGFβ substrate is utilised as a positive control and saliva from healthy people as a negative control.

A novel, non-invasive method for the early detection of oral cancer, the results that were presented came from the design of a reporter system model in yeast cells for the detection of TGFβ. This system can be used as a tool for quantification and spatial assessment of oral cancer by saliva sample.

Conclusions

The TGFβRII and Col2a1 plasmids, which contain the TGFβ and Col2a1 genes, were both successfully integrated into the eukaryotic expression system S. cerevisiae to produce recombinant protein that inhibits the GFP signal. This was done in the current study using a hybrid system of yeast. Since there isn't a different biological system at the moment, this transformant system is more advantageous and responds well to early disease detection in patients with oral cancer.

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Conflict of interest: All the authors declared that there is no conflict of interest in this research.

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