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Detection of Skin Squamous Cell Carcinoma using G Protein-Coupled Receptor 55 as a Biomarker

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Abstract

The purpose of this research was to investigate the presence of G Protein-Coupled Receptor 55 (GPR55) in patients suffering from skin Squamous Cell Carcinoma (SCC) compared to healthy people. In a comparative study the presence of GPR55 in plasma samples of patients using Human GPR55 ELISA kit were established. A total of 10 patients and 10 healthy people were included in the study. The study exhibited that GPR55 protein is present in the plasma of SCC patients. The results of the study suggest that the detection of GPR55 can be utilized as a biomarker for detection of SCC.

Keywords: Biomarker, Detection, GPR55, Protein, Skin Squamous Cell Carcinoma

1. Introduction

Cancer can be defined as a failure within the cells' communication network[1]. Skin cancer is currently the most common type of human cancer and of particular concern, its incidence is increasing at an astonishing rate. Epidemiological and molecular data strongly suggest that non-melanoma skin cancers are associated with excessive exposure to the Ultraviolet (UV) radiation in sunlight. Although stratospheric ozone blocks UVC (below 280 nm) radiation as well as part of UVB radiation (280–290 nm) from reaching the surface of the earth, UVB (290–315 nm) and UVA (315–400 nm) reach the surface of the earth and cause DNA damage, inflammation and erythema, sunburn, gene mutations, post-inflammatory immunosuppression, and eventually, skin cancer[2].

Cancer diagnosis and treatment are of great importance due to the high occurrence of the diseases and death rate. Survival of a cancer patient depends on early detection and thus developing technologies capable for sensitive and specific ways to detect cancer is a task for researchers. Existing cancer screening methods include: biopsy, endoscopy, CT scans, X-ray, fluorescent microscopy, ultrasound imaging and MRI[3]. However these traditional diagnostic ways, are not powerful detection methods at very early stages. At the same time screening methods are expensive and not readily available for many patients. Therefore, investigation about methods that are reliable, specific and sensitive for detecting cancers at early stages and are easily accessible as the first-line guidance is of outmost importance[3].

Biomarkers and nanotechnology, two new sciences in development of diagnostic methods are being extensively studied[3].

Researchers suggest some biomolecule such as G Protein-Coupled Receptor55 (GPR55) as biomarker for early detection of skin Squamous Cell Carcinoma (SCC)[4]. GPR55 is a seven-transmembrane G protein-coupled receptor identified in 1999[5]. Several groups reported that GPR55 is a possible novel type of cannabinoid receptor[6–8].

GPR55 expression has been reported in melanoma (skin cancer), human astrocytoma, lymphoblastoid cell lines, B lymphoblastoma[9,10], breast[10,11], ovary, prostate[12], brain, skin, cervix, liver, blood, pancreas[10].

Interestingly, the expression of GPR55 in human tumors correlates with their aggressiveness[10]. These data suggest that GPR55 expression and/or activation confers an oncogenic capability on cancer cells. Results obtained

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so far indicate that this capability increases cancer cells proliferative potential.

In the present study, it was shown that GPR55, at the protein level, is expressed in SCC patients in comparison with healthy people and that it could be utilized as a probable biomarker for detection of SCC.

2. Materials and Methods

Ten cases of skin SCC and ten cases of healthy people were nominated for detecting the presence of GPR55 in plasma.

Materials GPR55 ELISA Kit, including:

Reagents, assay plate (12 x 8 coated Microwells), Standard (Freeze dried), Biotin-antibody (100 x concentrate), HRP-avidin (100 x concentrate), Biotin-antibody Diluent, HRP-avidin Diluent, Sample Diluent, Wash Buffer (25 x concentrate), TMB Substrate, Stop Solution, Adhesive Strip (For 96 wells).

All reagents were kept to room temperature (25°C) for 30min before use. Fresh standards were prepared for each assay. The reagents were prepared as follows:

- Biotin-antibody (1x) - the vial was centrifuged before opening. 10 µl of Biotin-antibody was added to 990 µl of Biotin-antibody diluent.
- HRP-avidin (1x) - the vial was centrifuged before opening. 10 µl of HRP-avidin was added to 990 µl of HRP-avidin diluent.
- Wash Buffer (1x) - 20 ml of Wash Buffer Concentrate (25x) diluted with deionized water to prepare 500 ml of Wash Buffer (1x).
- Standards:

The standard vial was centrifuged at 8000 rpm for 30s and the content dissolved with 1.0 ml of sample diluent to produce a stock solution of 1500 pg/ml.

250 µl of sample diluent was pipetted into seven micro tubes and named (S0-S6). 250 µl of stock solution was pipetted into 6th tube. 250 µl of S6 was introduced to S5 and the procedure were done like previous step to have a series of standards. The undiluted standard name S7 served as concentrated standard (1500 pg/ml). Sample diluent (S0) served as the zero standards (0 pg/ml).

2.1 Sample Collection and Storage

Blood samples of eligible participants both treatment and control groups were collected in EDTA tubes by a trained pre-instructed nurse. Blood samples were centrifuged for 15 minutes at 1000 ×g at 4°C within 30 minutes of collection. Obtained plasma was aliquot and stored at -80°C for the experiments.

2.2 Study Design

This assay is based on the quantitative sandwich enzyme immunoassay technique. Specific antibody for GPR55 was pre-coated onto a micro-plate. Standards and samples were pipetted into the wells and any GPR55 present react with the immobilized antibody. By removing any unreacted and unbound substances, a specific biotin-conjugated antibody was added to the wells. Avidin conjugated Horseradish Peroxidase (HRP) was added after washing with wash water. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of GPR55 bound in the initial step. The color development was stopped and the intensity of the color was measured.

For treatment group patients referred to Emam Khomeini Hospital, Tehran, Iran, during the year 2014 for skin SCC were considered for eligibility in the study. The inclusion criterion was the existence of skin SCC.

Patients with other types of skin disorders or those who were receiving other chemical drugs at the time of admission were excluded.

For control group 10 healthy people were included in the study. Patients admitted to Emam Khomini Hospital were asked to be included in the study provided they met the eligibility criteria and signed the informed consent form before to be included in the study.

2.3 Ethical Considerations

Before being included in the study, the eligible participants accepted to sign the informed consent. Each participant was allowed to withdraw from the study whenever they desired. All steps for collecting blood samples were provided for the participants free of charge. Finally, all of the patients' information was classified and the findings were reported anonymously.

2.4 Study Procedure

100µl of each standard was introduced in eight individual pre-coated microplate wells. 100µl of plasma samples each individual member of patient and control group were added per well. The filled wells were covered with an adhesive strip and incubated for 2 hours at 37°C.

After incubation the liquid of each well was decanted. 100µl of diluted biotin-antibody was added per well, covered and incubated for a 1 hour at 37°C.

Following incubation each well was aspirated and washed three times with wash buffer (200µl) using a multi-channel pipette and let it stand for 2 minutes. Then any remaining wash buffer was removed. 100µl of HRP-avidin (1x) was add to each well and covered with adhesive strip and incubated for 1 hour at 37°C.

Then the aspiration/wash process was repeated for five times. 90µl of TMB substrate was added per well, incubated for 20 minutes at 37°C and 50µl of stop solution was added. The optical density of each well was determined within 5 minutes, using a microplate reader set to 450 nm.

The diagnoses of all biopsies performed on study participants during the trial were reviewed centrally by one of two reference study dermatopathologists, whose diagnosis was considered definitive for study purposes. For this purpose the samples were fixed first and colored with Hematoxylin and Eosin Stain (H and E). The staining method involves application of Hematoxylin, which is a complex formed from aluminium ions and oxidized Hematoxylin. This material colors nucleus blue. The nuclear staining is followed by counterstaining with eosin, which colors eosinophilic other structures in various shades of red and pink. After preparation, they were viewed through optic microscope with magnitude of 400.

3. Results

Optical density of samples was measured at 450 nm. GPR55 was predominantly found in the treatment's group samples due to more absorbance in this wavelength.

Figure 1 illustrate standard curve. Normal amount of GPR55 in control group plasma was between 20-80 pg/ml.

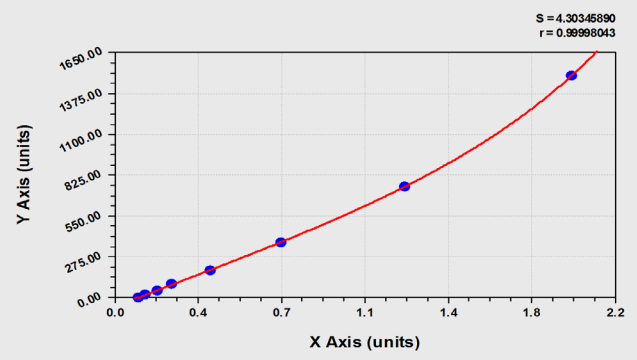

Figure 1. Standard curve.

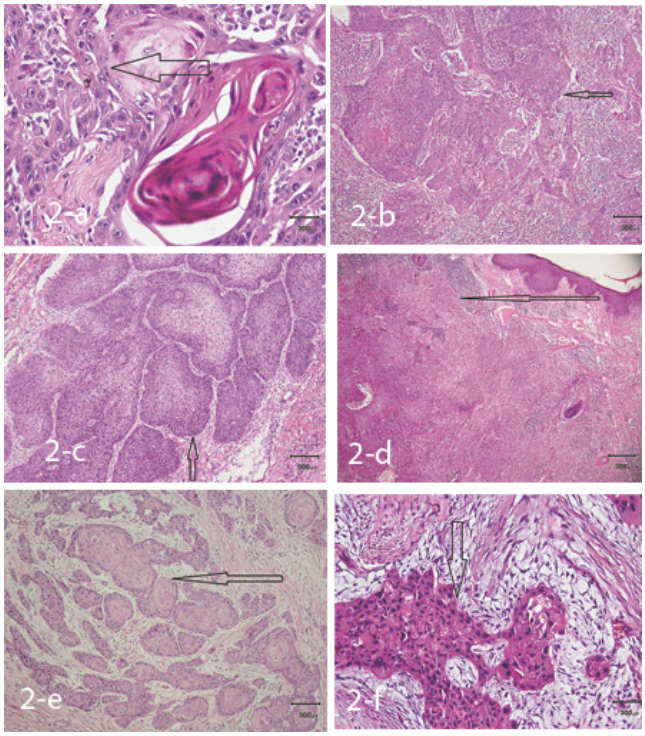

Figure 2(a-f). Abnormal SCC cells of six different patients.

The amount of GPR55 in treatment group was between 825-1100 pg/ml.

Pathology reports exhibited that patients in treatment group suffer from skin SCC disease. The abnormal SCC cells of six different patients have been shown in Figures 2(a-f).

4. Discussion

This study was conducted aiming to explore the significance of the expression of GPR55 protein in plasma of patients suffering from skin SCC as compared with healthy people. A recent study has indicated that GPR55 gene over expression in SCC patients can be detected[4].

It is well known that over expression of a specific protein can be symbol of a disease. GPR55 is over expressed in human skin tumor. Recent data suggest that GPR55 may be part of the molecular controlling tumor growth. Research point that GPR55 has a pivotal role in skin tumor development and it is suggested that this receptor may be used as a new therapeutic target and potential biomarker in skin cancer management[4].

In this study we investigate GPR55 as a probable biomarker for early detection of skin SCC.

GPR55 in the patients' plasma would bind with antibody. TMB is a solution of 3, 3', 5, 5'- tetra methylbenzidine which react with Horseradish Peroxidase (HRP) enzyme and create a blue color. The intensity of color depends on the GPR55 concentration in the samples. By using stop solution and acidifying reaction the color turns to yellow.

Although GPR55 gene has been evaluated in previous studies as biomarker[10–14], in none of the previous studies the role of GPR55 protein as a biomarker for detecting SCC have been utilized. This work was the first comparative study investigating the presence of GPR55 protein in the plasma of patients suffering from SCC against healthy people. The results from this study were further confirmed by pathology reports.

Early detection of SCC employing conclusive, sensitive and specific methods is a key to survival of a cancer patient. It appears that the presence of GPR55 protein can be an effective biomarker in SCC patients. The present study suggests that GPR55 could be used as a biomarker for SCC disorder. These results combined with other non-invasive early detection methods could open a new horizon for the detection and consequent treatment of SCC patients.

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