ANALYTICAL METHOD FOR COMMON AND SPECIFIC CHARACTERIZATION OF SKIN CARCINOGENESIS BY FTIR MICROSPECTROSCOPY

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

The present invention is directed towards providing an optical method for non-destructive characterization of skin carcinogenesis in benign, premalignant and malignant skin tumourous tissues based on Fourier transform infrared (FTIR) microspectroscopy. More specifically, the invention provides with an analytical method for simultaneous observation and further characterization of intra- and inter-molecular interactions for nucleic acids and proteins, commonly and specifically expressed in IR spectra of each type of pathology, that also finds use for indication of the grade of neoplastic activity in cells in human skin tumourous tissues.

2. Prior Art

Publications and other reference materials referred to herein, including reference cited therein, are incorporated herein by reference in their entirety and are numerically referenced in the following text and respectively grouped in the appended Bibliography, which immediately preceds the claims.

In many countries skin cancer affects more people than all other cancers combined [1], but there is not yet enough understanding about the molecular, cellular and tissue changes that occur during skin carcinogenesis, that will have impact on clinical diagnosis, monitoring and treatment of skin cancer.

For many years the mouse skin model provided a possibility to study multistage carcinogenesis mechanisms [2], but without direct relevance of this model system to human skin cancers.

Up-to-date literature describes skin cancer development is, in part, under genetic control, and transcription factors (TFs), which are sequence-specific DNA-binding proteins, that control transcriptional activation or regression and thus control cell growth, being important in this process [3].

However, existing methods do not permit studying mechanisms of cancerogenesis directly on human beings and do not answer to all existing questions at present.

For better understanding of molecular and cellular mechanisms involved in skin cancer development, there is a need for a method to directly characterize carcinogenesis from

hyperplasia to invasive cancer, from one type of cancer to another type of cancer, from one patient to the group of patients with the same pathology.

Skin tissue IR microspectroscopy technique is a tool for spectroscopic evaluation of unstained tissue of skin biopsies. It is an objective method for the analysis of skin tissue section that uses the chemical composition of the tissue as an indicator for healthy or pathological state of the cells in the tissue. IR spectral information objectively and quantitatively determines variations in the chemical composition of skin cells and tissue, that can be further "converted" to medical knowledge. It is a sensitive tool for studying isolated biomolecules alone and in interactions.

Feasibility of application of IR microspectroscopy for spectral characterization of the most common skin precancers and cancers has been already introduced in the 900-1700 cm^{-1} region, revealing the most visible changes related to protein conformation and nucleic acid bases, that in general showed modifications and enhancement with progression to malignancy in that region [4].

Detailed alignment of the peaks for nucleic acids and proteins with spectral variations in IR spectra from skin epidermal cancers described appearance of DNA peak at 965 cm^{-1}; the multiplet (DNA/RNA) at 1055 cm^{-1}; DNA/RNA triad peaks at 1071 cm^{-1}, 1084/1085 cm^{-1}, 1095 cm^{-1}; at 1245 cm^{-1} as a combination for DNA and amide III; a number of non-specific (non-descriptive) proteins at 1310 cm^{-1}, 1390 cm^{-1} and 1450 cm^{-1}; the amide II vibration at 1540 cm^{-1}; and the amide I vibration at 1650 cm^{-1} [5]. Nevertheless, the problem has not been yet addressed towards common and specific characterization of carcinogenesis in human skin tumours by FTIR microspectroscopy.

Therefore, it is another purpose of this invention to provide an optical analytical method for objective evaluation of intra- and inter-molecular interactions for nucleic acids and proteins expressed in infrared (IR) spectra of epidermal skin benign, premalignant and malignant tumours towards understanding the molecular, cellular and tissue changes that occur during skin carcinogenesis.

SUMMARY OF THE INVENTION

In a first aspect, the invention provides a method of using FTIR microspectroscopy for characterization of common and specific skin carcinogenesis in human biopsied tumourous tissue samples. The sample can be comprised of normal, benign, premalignant or malignant cells on a tissue sample. The invented method is based on simultaneous characterization of intra- and inter-molecular interactions for nucleic acids

and proteins by means of their expressed interacting activity levels in infrared (IR) spectra of benign, premalignant and malignant skin tumours, in comparison to IR spectra from healthy, i.e. normal/unchanged, skin tissue. More particularly, provided method allows to describe common and specific features of skin carcinogenesis for each type of tumour and between different types of tumours, to assess the activity of neoplastic cells in each patient and between the patients by FTIR microspectroscopy.

Skin epidermal carcinogenesis is a multi-stage process, that involves at least 3 mechanistically distinct steps - initiation, promotion and progression. Skin malignant tumour generally develops in the epidermis. Most common malignant skin tumours are are basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma (MM), that have been used as examples of malignant skin tumours for the user of invention. To further provide the user with the invention steps, Bowen's disease (BD), has been chosen as an example of premalignant skin tumour, well-known as intraepidermal carcinoma *in situ*. Benign compound nevus (BCN) has been chosen as a common example of benign skin tumour in the presented invention.

The method can be carried out manually and comprises the following steps:

i. obtaining FTIR spectra from a multitude of pathological sites on a sample to obtain a multitude of measurements for each sample

ii. determining the specified wavenumber region between 900 cm^{-1} and 1300 cm^{-1} for nucleic acids and the specified wavenumber region between 1300 cm^{-1} and 1700 cm^{-1} for proteins in each measured IR spectrum

iii. normalizing each of IR spectrum to amide I peak

iv. averaging epidermal measurements to obtain an average spectrum for the sample

In the above method, the biopsied tissue samples are prepared for measurements by using FTIR microspectroscopy by the following steps:

i. strictly sequential 2 sample cuts having a thickness of 6 micrometers

ii. staining 1 sample cut with hematoxilin and eosin for histopathological evaluation

iii. air-drying 1 sample cut on CaF_2 slide glass for collection of FTIR spectra

Although skin tissue is used throughout this description as the representative tissue, it should be understood that the invention method is not limited to measuring epidermal skin tissue and can be exploited with other organ tissues, as will be apparent to the skilled person.

All the above and other characteristics and advantages of the invention will be further described.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be further explained through description of preferred embodiments. All the FTIR microscopy measurements were performed using a JEOL Co. (Tokyo, Japan) FT-IR spectrometer, a model IR-MAU200. Before each measurement, a calibration was performed using a sample provided with the instrument by the manufacturer and proper operating conditions of FTIR microspectrometer was confirmed.

The data used in the examples below is derived from tissue specimen from the biopsy archive of the Department of Dermatology, Tokushima University School of Medicine, Tokushima, Japan, and a courtesy of Dr. T. Ikehara from the Department of Orthopedics, Tokushima University School of Medicine, Tokushima, Japan.

The tissue samples used for FT-IR microspectroscopy were in the dry state, while the corresponding slides observed by light microscope were stained with hematoxilin and eosin for the identification of the tissue structure. The aperture size used in the measurements was 25 over 25 micrometers.

Initially, the background spectrum was collected. After the measurement site was chosen using the visible light, the microscope was changed to IR mode. The number of co-added scans were increased to 127 to achieve high signal to noise ratio. The measured spectra covered the wavenumber range between 800 cm^{-1} and 4000 cm^{-1}, at a resolution of 4 cm^{-1}.

A database of having totally measured 198 spectra in the epidermis, both pathological and non-pathological, was created from 13 patients with malignant skin tumours: BCC (6 patients), SCC (4 patients), MM (3 patients); from 3 patients with premalignant skin tumours: BD (3 patients); from 4 patients with benign skin tumours: BCN (4 patients); and from 4 healthy subjects.

The invention method included the following steps for determination of the activity levels for nucleic acids and proteins in the IR spectra of BCC, SCC, MM, BD and BCN, in comparison to IR spectra of normal skin from healthy subjects:

- calculation of mean values of each determined nucleic acids peak;
- calculation of mean values of each determined protein (specific and non-specific) peak;
- calculation of the intensities of all determined nucleic acids peaks in the 900-1300 cm^{-1} region with the intensity of the peak at 1245 cm^{-1} by calculating the following intensity peaks ratios: I(965)/I(1245), I(1055)/I(1245), I(DNA/RNA triad max peak)/I(1245);
- calculation of mean values and/or the intensities of all determined peaks for specific and nonspecific proteins in the 1300-1700 cm^{-1} region with the intensity of amide I peak at 1650 cm^{-1} by calculating the following peaks ratios: I(1310)/I(1650), I(1390)/I(1650), I(1450)/I(1650), I(1540)/I(1650);
- calculation of the level of activity of DNA-RNA vs. DNA-protein interactions by the following ratio: I(sum mean DNA/RNA triad peaks)/I(1245);
- comparison of the level of activity of multiplet (DNA/RNA) with the level(s) of activity of the peaks in DNA/RNA triad to observe DNA-RNA and DNA-DNA interactions in spectral patterns of sequentially expressed nucleic acids at 965 cm^{-1}, 1055 cm^{-1} and/or 1071,1084/1085,1095 cm^{-1};
- comparison of the level of activity of specific and non-specific proteins to observe DNA-protein and protein-protein interactions in spectral patterns of sequentially expressed proteins at 1245 cm^{-1}, 1310 cm^{-1}, 1390 cm^{-1}, 1450 cm^{-1}, 1540 cm^{-1} and 1650 cm^{-1}.

Below is a summary of the data employed from the patients with BCC, SCC, MM, BD, BCN and healthy subjects, provided in examples.

INTRA- AND INTER-MOLECULAR INTERACTIONS FOR NUCLEIC ACIDS EXAMPLES

EXAMPLE 1
BCC

Spectral data epidermally measured from 6 patients with BCC on nucleic acids peaks mean values (965 cm^{-1}, 1055 cm^{-1}, the most prominent peak in DNA/RNA triad peaks),

the intensity ratios and proportional ratios (sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}).

3 BCC spectral patterns of nucleic acids in 6 patients, based on expressed mean values vs. proportional ratios:

- *965 < 1055 < DNA/RNA triad max peak*
- *965 < 1055 > DNA/RNA triad max peak*
- *965 = 1055 < DNA/RNA triad max peak*

1)	0.35 < 0.44 < 0.45	vs.	[1:1]
2)	0.31 < 0.43 < 0.48	vs.	[1:1]
3)	0.35 < 0.42 > 0.40	vs.	[1:1]
4)	0.11 < 0.19 > 0.18	vs.	[0.8:1]
5)	0.08 < 0.14 < 0.16	vs.	[0.8:1]
6)	0.09 = 0.09 < 0.23	vs.	[0.8:1]

3 BCC spectral patterns of nucleic acids in 6 patients, based on calculated intensity ratios vs. proportional ratios:

- *I (965)/I(1245) < I(1055)/I(1245) < I (DNA/RNA triad max peak)/I(1245)*
- *I (965)/I(1245) < I(1055)/I(1245) > I (DNA/RNA triad max peak)/I(1245)*
- *I (965)/I(1245) = I(1055)/I(1245) < I (DNA/RNA triad max peak)/I(1245)*

1)	0.75 < 0.94 < 0.96	vs.	[1:1]
2)	0.71 < 0.98 < 1.09	vs.	[1:1]
3)	0.88 < 1.05 > 1.00	vs.	[1:1]
4)	0.50 < 0.87 > 0.82	vs.	[0.8:1]
5)	0.38 < 0.67 < 0.76	vs.	[0.8:1]
6)	0.45 = 0.45 < 1.15	vs.	[0.8:1]

EXAMPLE 2

SCC

Spectral data epidermally measured from 4 patients with SCC on nucleic acids peaks mean values (965 cm^{-1}, 1055 cm^{-1}, the most prominent peak in DNA/RNA triad peaks),

the intensity ratios and proportional ratios (sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}).

4 SCC spectral patterns of nucleic acids in 4 patients, based on expressed mean values vs. proportional ratios:

- *965 < 1055 > DNA/RNA triad max peak* vs. [1:1]
- *965 < 1055 < DNA/RNA triad max peak* vs. [0.9:1]
- *965 > 1055 < DNA/RNA triad max peak* vs. [0.7:1]
- *965 > (no peak expression) < DNA/RNA triad max peak* vs. [0.7:1]

1) 0.60 < 0.66 > 0.65 vs. [1:1]
2) 0.26 < 0.30 < 0.35 vs. [0.9:1]
3) 0.19 > 0.13 < 0.16 vs. [0.7:1]
4) 0.07 > (-) < 0.12 vs. [0.7:1]

3 SCC spectral patterns of nucleic acids in 4 patients, based on calculated intensity ratios vs. proportional ratios:

- *I (965)/I(1245) < I(1055)/I(1245) > I (DNA/RNA triad max peak)/I(1245)*
- *I (965)/I(1245) > I(1055)/I(1245) < I (DNA/RNA triad max peak)/I(1245)*
- *I (965)/I(1245) > (no peak expression) < I (DNA/RNA triad max peak)/I(1245)*

1) 0.91 < 1.00 > 0.99 vs. [1:1]
2) 0.70 < 0.81 < 0.88 vs. [0.9:1]
3) 0.41 < 0.77 > 0.71 vs. [0.7:1]
4) 0.91 > (-) < 0.76 vs. [0.7:1]

EXAMPLE 3

MM

Spectral data epidermally measured from 3 patients with MM on nucleic acids peaks mean values (965 cm^{-1}, 1055 cm^{-1}, the most prominent peak in DNA/RNA triad peaks), the intensity ratios and proportional ratios (sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}).

3 MM spectral patterns of nucleic acids in 3 patients, based on expressed mean values

vs. proportional ratios:

- *965 > 1055 < DNA/RNA triad max peak*
- *965 < 1055 < DNA/RNA triad max peak*
- *965 > (no peak expression) < DNA/RNA triad max peak*

1) 0.19 > 0.14 < 0.27 vs. [0.8:1]
2) 0.25 > (-) < 0.35 vs. [0.7:1]
3) 0.23 < 0.26 < 0.30 vs. [0.8:1]

3 MM spectral patterns of nucleic acids in 3 patients, based on calculated intensity ratios vs. proportional ratios:

- *I (965)/I(1245) < I(1055)/I(1245) < I (DNA/RNA triad max peak)/I(1245)*
- *I (965)/I(1245) > I(1055)/I(1245) < I (DNA/RNA triad max peak)/I(1245)*
- *I (965)/I(1245) > (no peak expression) < I (DNA/RNA triad max peak)/I(1245)*

1) 0.61 < 0.69 < 0.79 vs. [0.8:1]
2) 0.63 > 0.47 < 0.90 vs. [0.8:1]
3) 0.52 > (-) < 0.73 vs. [0.7:1]

EXAMPLE 4
BOWEN'S DISEASE (BD)

Spectral data epidermally measured from 3 patients with BD on nucleic acids peaks mean values (965 cm^{-1}, 1055 cm^{-1}, the most prominent peak in DNA/RNA triad peaks), the intensity ratios and proportional ratios (sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}).

2 BD spectral patterns of nucleic acids in 3 patients, based on expressed mean values:

- *965 < 1055 < DNA/RNA triad max peak*
- *(no peak expression) (no peak expression) DNA/RNA triad max peak*

1) (-) (-) 0.07 vs. [0.7:1]
2) 0.09 < 0.15 < 0.18 vs. [0.8:1]
3) 0.23 < 0.26 < 0.29 vs. [0.9:1]

2 BD spectral patterns of nucleic acids in 3 patients, based on calculated intensity ratios:

- *I (965)/I(1245) < I(1055)/I(1245) < I(DNA/RNA triad max peak)/I(1245)*
- *(no peak expression) (no peak expression) I (DNA/RNA triad max)*

1) (-) (-) 0.64 vs. [0.7:1]
2) 0.41 < 0.68 < 0.82 vs. [0.8:1]
3) 0.70 < 0.79 < 0.88 vs. [0.9:1]

EXAMPLE 5

BENIGN COMPOUND NEVUS (BCN)

Spectral data epidermally measured from 4 patients with BCN on nucleic acids peaks mean values (965 cm^{-1}, 1055 cm^{-1}, the most prominent peak in DNA/RNA triad peaks), the intensity ratios and proportional ratios (sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}).

1 BCN spectral pattern of nucleic acids in 4 patients, based on expressed mean values vs. proportional ratios:

- *965 < 1055 < DNA/RNA triad max peak*

1) 0.15 < 0.22 < 0.23 vs. [0.8:1]
2) 0.15 < 0.19 < 0.22 vs. [0.7:1]
3) 0.18 < 0.24 < 0.27 vs. [0.9:1]
4) 0.12 < 0.17 < 0.19 vs. [0.7:1]

1 BCN spectral pattern of nucleic acids in 4 patients, based calculated intensity ratios vs. proportional ratios:

- *I (965)/I(1245) < I(1055)/I(1245) < I(DNA/RNA triad max peak)/I(1245)*

1) 0.48 < 0.71 < 0.78 vs. [0.8:1]
2) 0.47 < 0.59 < 0.72 vs. [0.7:1]
3) 0.56 < 0.75 < 0.84 vs. [0.9:1]
4) 0.48 < 0.68 < 0.76 vs. [0.7:1]

EXAMPLE 6

HEALTHY SKIN (HS)

Spectral data epidermally measured from 4 healthy subjects with normal skin on nucleic acids peaks mean values (965 cm^{-1}, the most prominent peak in DNA/RNA triad peaks), the intensity ratios and proportional ratios (sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}).

HS spectral pattern of nucleic acids, based on expressed mean values vs. proportional ratios:

- *965 > (no peak expression) < DNA/RNA triad max peak*

1) 0.15 > (-) < 0.27	vs.	[0.5:1]
2) 0.24 > (-) < 0.47	vs.	[0.5:1]
3) 0.17 > (-) < 0.22	vs.	[0.7:1]
4) 0.22 > (-) < 0.30	vs.	[0.7:1]

HS spectral pattern of nucleic acids, based on calculated intensity ratios vs. proportional ratios:

- *I (965)/I (1245) > (no peak expression) < I (DNA/RNA triad max peak)/I (1245)*

1) 0.41 > (-) < 0.75	vs.	[0.5:1]
2) 0.51 > (-) < 1.00	vs.	[0.5:1]
3) 0.38 > (-) < 0.49	vs.	[0.7:1]
4) 0.51 > (-) < 0.70	vs.	[0.7:1]

RESULTS

Based on the presented Examples 1-5, the activity level of the multiplet at 1055 cm^{-1} strongly correlated with the activity level of the most prominent peak expressed in DNA/RNA triad (1071 cm^{-1}, 1084/1085 cm^{-1}, 1095 cm^{-1}) in 5 out of 6 patients with BCC, in 3 out of 4 patients with SCC and only in 1 out of 3 patients with MM, as well in all patients with BCN and BD. However, calculated proportional ratios differed for mean values and the intensity ratios between malignant and benign/premalignant skin tumours, being higher in malignant skin tumours.

Pattern recognition on DNA-RNA and DNA-DNA interactions as *I(965) < I(1055) < I*

(DNA/RNA triad max peak) were the most clearly observed in all 6 patients with BCC, independent on expressed activity levels of all peaks of nucleic acids. The same pattern recognition on DNA-RNA and DNA-DNA interactions were seen in all patients with BD and BCN, independently on the activity levels expressed by all peaks of nucleic acids.

Pattern recognition on DNA-RNA and DNA-DNA interactions as *I(965) < I(1055) < I (DNA/RNA triad max peak)* was clearly seen in 3 SCC patients with the highest activity levels of the multiplet at 1055 cm^{-1}.

In 2 patients with MM pattern recognition on DNA-RNA and DNA-DNA interactions as *I(965) < I(1055) < I (DNA/RNA triad max peak)* was unique and not seen in any other pathologies, with the lowest activity level of the multiplet.

In 4 healthy subjects pattern recognition on DNA-RNA and DNA-DNA interactions as *I(965) < I(1055) < I(DNA/RNA triad max peak)* was different from all measured above pathologies, with no expression of the multiplet at 1055 cm^{-1} in the epidermis.

INTRA- AND INTER-MOLECULAR INTERACTIONS FOR PROTEINS

EXAMPLES

Spectral data epidermally measured from 6 patients with BCC, 4 patients with SCC, 3 patients with MM, 3 patients with BD, 4 patients with BCN, 4 healthy subjects with normal skin on proteins peaks mean values and calculated intensities (1245 cm^{-1}, 1310 cm^{-1}, 1390 cm^{-1}, 1450 cm^{-1}, 1540 cm^{-1}, 1650 cm^{-1}), in correlation to proportional ratios (sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}).

EXAMPLE 1

BCC

2 BCC spectral patterns of proteins from 6 patients with BCC, based on expressed mean values and/or calculated intensity ratios:

- 1245 = 1310 < 1390 = 1450 < 1540 < 1650
- 1245 > 1310 = 1390 = 1450 < 1540 < 1650

1) BCC: 0.5 > 0.4 = 0.4 = 0.4 < 0.7 < 1.0 vs. [1:1]
2) BCC: 0.5 > 0.4 = 0.4 = 0.4 < 0.7 < 1.0 vs. [1:1]

3) BCC: 0.4 = 0.4 < 0.5 = 0.5 < 0.8 < 1.0 vs. [1:1]

4) BCC: 0.2 = 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.8:1]

5) BCC: 0.2 = 0.2 < 0.3 = 0.3 < 0.8 < 1.0 vs. [0.8:1]

6) BCC: 0.2 = 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.8:1]

EXAMPLE 2

SCC

3 SCC spectral patterns of proteins from 4 patients with SCC, based on expressed mean values and/or calculated intensity ratios:

- 1245 = 1310 = 1390 = 1450 < 1540 < 1650
- 1245 > 1310 < 1390 = 1450 < 1540 < 1650
- 1245 = 1310 < 1390 = 1450 < 1540 < 1650

1) SCC: 0.7 > 0.6 < 0.7 = 0.7 < 0.9 < 1.0 vs. [1:1]

2) SCC: 0.4 = 0.4 = 0.4 = 0.4 < 0.7 < 1.0 vs. [0.9:1]

3) SCC: 0.2 = 0.2 < 0.3 = 0.3 < 0.6 < 1.0 vs. [0.7:1]

4) SCC: 0.2 = 0.2 = 0.2 = 0.2 < 0.6 < 1.0 vs. [0.7:1]

EXAMPLE 3

MM

2 MM spectral patterns of proteins from 3 patients with MM, based on expressed mean values and/or calculated intensity ratios:

- 1245 = 1310 < 1390 = 1450 < 1540 < 1650
- 1245 > 1310 < 1390 = 1450 < 1540 < 1650

1) MM: 0.4 = 0.4 < 0.5 = 0.5 < 0.8 < 1.0 vs. [0.8:1]

2) MM: 0.3 = 0.3 < 0.4 = 0.4 < 0.8 < 1.0 vs. [0.8:1]

3) MM: 0.5 > 0.4 < 0.5 = 0.5 < 0.8 < 1.0 vs. [0.7:1]

EXAMPLE 4

SCC vs. BD

1 BD spectral pattern of proteins from 3 patients with BD, based on expressed mean values and/or calculated intensity ratios:

- 1245 = 1310 < 1390 = 1450 < 1540 < 1650

3 SCC spectral patterns of proteins from 4 patients with SCC, based on expressed mean values and/or calculated intensity ratios:

- 1245 = 1310 = 1390 = 1450 < 1540 < 1650
- 1245 > 1310 < 1390 = 1450 < 1540 < 1650
- 1245 = 1310 < 1390 = 1450 < 1540 < 1650

1) SCC: 0.7 > 0.6 < 0.7 = 0.7 < 0.9 < 1.0 vs. [1:1]
2) SCC: 0.4 = 0.4 = 0.4 = 0.4 < 0.7 < 1.0 vs. [0.9:1]
3) BD: 0.3 = 0.3 < 0.4 = 0.4 < 0.8 < 1.0 vs. [0.9:1]
4) BD: 0.2 = 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.8:1]
5) SCC: 0.2 = 0.2 < 0.3 = 0.3 < 0.6 < 1.0 vs. [0.7:1]
6) SCC: 0.2 = 0.2 = 0.2 = 0.2 < 0.6 < 1.0 vs. [0.7:1]
7) BD: 0.1 = 0.1 < 0.2 = 0.2 < 0.6 < 1.0 vs. [0.7:1]

EXAMPLE 5

MM vs. BCN

3 BCN spectral patterns of proteins from 4 patients with BCN, based on expressed mean values and/or calculated intensity ratios:

- 1245 = 1310 < 1390 = 1450 < 1540 < 1650
- 1245 > 1310 < 1390 < 1450 < 1540 < 1650
- 1245 > 1310 < 1390 = 1450 < 1540 < 1650

2 MM spectral patterns of proteins from 3 patients with MM, based on expressed mean values and/or calculated intensity ratios:

- 1245 = 1310 < 1390 = 1450 < 1540 < 1650
- 1245 > 1310 < 1390 = 1450 < 1540 < 1650

1) BCN: 0.3 > 0.2 < 0.3 < 0.4 < 0.7 < 1.0 vs. [0.9:1]

2) BCN: 0.3 > 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.8:1]
3) BCN: 0.3 > 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.7:1]
4) BCN: 0.3 = 0.3 < 0.4 = 0.4 < 0.8 < 1.0 vs. [0.7:1]

1) MM: 0.4 = 0.4 < 0.5 = 0.5 < 0.8 < 1.0 vs. [0.8:1]
2) MM: 0.3 = 0.3 < 0.4 = 0.4 < 0.8 < 1.0 vs. [0.8:1]
3) MM: 0.5 > 0.4 < 0.5 = 0.5 < 0.8 < 1.0 vs. [0.7:1]

Example 6

ALL MEASURED PATHOLOGIES

1) BCC: 0.5 > 0.4 = 0.4 = 0.4 < 0.7 < 1.0 vs. [1:1]
2) BCC: 0.5 > 0.4 = 0.4 = 0.4 < 0.7 < 1.0 vs. [1:1]
3) BCC: 0.4 = 0.4 < 0.5 = 0.5 < 0.8 < 1.0 vs. [1:1]
4) SCC: 0.7 > 0.6 < 0.7 = 0.7 < 0.9 < 1.0 vs. [1:1]

5) SCC: 0.4 = 0.4 = 0.4 = 0.4 < 0.7 < 1.0 vs. [0.9:1]
6) BD : 0.3 = 0.3 < 0.4 = 0.4 < 0.8 < 1.0 vs. [0.9:1]
7) BCN: 0.3 > 0.2 < 0.3 < 0.4 < 0.7 < 1.0 vs. [0.9:1]

8) MM: 0.4= 0.4 < 0.5 = 0.5 < 0.8 < 1.0 vs. [0.8:1]
9) MM: 0.3 = 0.3 < 0.4 = 0.4 < 0.8 < 1.0 vs. [0.8:1]
10) BCC: 0.2 = 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.8:1]
11) BCC: 0.2 = 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.8:1]
12) BCC: 0.2 = 0.2 < 0.3 = 0.3 < 0.8 < 1.0 vs. [0.8:1]
13) BD: 0.2 = 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.8:1]
14) BCN: 0.3 > 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.8:1]

15) SCC: 0.2 = 0.2 < 0.3 = 0.3 < 0.6 < 1.0 vs. [0.7:1]
16) SCC: 0.2 = 0.2 = 0.2 = 0.2 < 0.6 < 1.0 vs. [0.7:1]
17) MM: 0.5 > 0.4 < 0.5 = 0.5 < 0.8 < 1.0 vs. [0.7:1]
18) BD: 0.1 = 0.1 < 0.2 = 0.2 < 0.6 < 1.0 vs. [0.7:1]
19) BCN: 0.3 > 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.7:1]
20) BCN: 0.3 = 0.3 < 0.4 = 0.4 < 0.8 < 1.0 vs. [0.7:1]

RESULTS

Presented data on DNA-protein interactions were mostly expressed between intensities of DNA/RNA triad peaks and non-descriptive proteins at 1310 cm^{-1}, 1390 cm^{-1} and 1450 cm^{-1}, that were in agreement between BCC and SCC, but not in MM.

Protein-protein interactions were similar between the patients, indicating the grade of activity in cells in tissues.

Among the patients with BCC and SCC the levels of non-descriptive proteins generally differed between 0.2 and 0.7. In 3 patients with BCC *I(sum mean DNA/RNA triad peaks) / I(1245)* was [1:1] and their levels of non-descriptive proteins were stable at the level of 0.4-0.5. Similar levels were observed in 3 patients with MM, but when their *I(sum mean DNA/RNA triad peaks)/I(1245)* were [0.7:1] and [0.8:1]. In all patients with SCC the levels of non-descriptive proteins were the most sensitive to clearly indicate the activity of *I(sum mean DNA/RNA triad peaks) / I(1245).*

Protein-protein interactions were observed by the correlation between *I(amide II) / I (amide I)* and mean values of the peaks at 1310 cm^{-1}, 1390 cm^{-1} and 1450 cm^{-1}.

Protein-protein interactions were similar between skin tumours, generally indicating low, medium and high activity levels in cells in tissues.

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CLAIMS

1. A method of spectral pattern analysis of sequentially expressed nucleic acids and proteins in FTIR spectra from benign, premalignant and malignant skin tumourous tissues for characterization of multi-stage skin epidermal carcinogenesis on a biopsied tissue sample of a human, comprising of:

(i) recognizing the spectral pattern of sequentially expressed nucleic acids to DNA (965 cm^{-1}), DNA/RNA (1055 cm^{-1}), DNA/RNA triad (1071,1084/1085,1095 cm^{-1}) from determined precise order of expressed nucleic acid peaks in FTIR spectra corresponding to common expression of the nucleic acid with carcinogenesis of the tissue to be detected in benign skin tumourous tissues with reference to benign compound nevus (BCN), or premalignant skin tumourous tissues with reference to Bowen's disease (BD), or malignant skin tumourous tissues with reference to basal cell carcinoma (BCC), or malignant skin tumourous tissues with reference to squamous cell carcinoma (SCC), or malignant skin tumourous tissues with reference to malignant melanoma (MM);

(ii) recognizing the spectral pattern of sequentially expressed nucleic acids from detected expressed nucleic acids mean peaks values and their calculated intensities in FTIR spectra corresponding to specific expression of the nucleic acid with carcinogenesis of the tissue to be detected in benign, premalignant or malignant skin tumourous tissues with reference to BCN, BD, MM, SCC or BCC, wherein the spectral patterns of sequentially expressed nucleic acids for

benign skin tumourous tissues is

(965) < (1055) < (1071,1084/1085,1095),

premalignant skin tumourous tissues is

(965) < (1055) < (1071,1084/1085,1095),

or

(no peak expression) (no peak expression) (1071,1084/1085,1095),

malignant skin tumourous tissues is

(965) > (no peak expression) < (1071,1084/1085,1095), or

(965) > (1055) < (1071,1084/1085,1095), or

(965) = (1055) < (1071,1084/1085,1095), or

(965) < (1055) < (1071,1084/1085,1095), or

(965) < (1055) > (1071,1084/1085,1095);

(iii) calculating proportional ratio of mean sum of peak values in DNA/RNA triad to DNA/amide III peak value at 1245 cm^{-1},

wherein characteristic range for

benign skin tumourous tissues is [0.7:1] - [0.9:1],

premalignant skin tumourous tissues is [0.7:1] - [0.9:1],

malignant skin tumourous tissues is [0.7:1] - [1:1];

(iv) recognizing the spectral pattern of sequentially expressed proteins to amide III at 1245 cm^{-1}, to non-specific proteins at 1310 cm^{-1}, 1390 cm^{-1} and 1450 cm^{-1}, to amide II at 1540 cm^{-1} and to amide I at 1650 cm^{-1} from detected their expressed mean peaks values and calculated intensities in FTIR spectra corresponding to specific expression of the proteins with carcinogenesis of the tissue to be detected in benign skin tumourous tissues with reference to BCN, or premalignant skin tumourous tissues with reference to BD, or malignant skin tumourous tissues with reference to BCC, or malignant skin tumourous tissues with reference to SCC, or malignant skin tumourous tissues with reference to MM, wherein

the spectral patterns of sequentially expressed proteins for

benign skin tumourous tissues is

(1245) > (1310) < (1390) < (1450) < (1540) < (1650), or

(1245) = (1310) < (1390) = (1450) < (1540) < (1650),

premalignant skin tumourous tissues is

(1245) = (1310) < (1390) = (1450) < (1540) < (1650),

malignant skin tumourous tissues is

(1245) = (1310) = (1390) = (1450) < (1540) < (1650), or

(1245) > (1310) = (1390) = (1450) < (1540) < (1650), or

(1245) = (1310) < (1390) = (1450) < (1540) < (1650), or

(1245) > (1310) < (1390) = (1450) < (1540) < (1650);

(v) correlating said spectral patterns of sequential expression of nucleic acids and proteins in FTIR spectra with said proportional ratio to characterize the expression of nucleic acids and proteins with carcinogenesis of the tissue to be detected;

(vi) comparing the sequential expression of nucleic acids and proteins in recognized spectral patterns obtained on a biopsied tissue sample to identify differences corresponding to specific expression of nucleic acids and proteins with carcinogenesis of the tissue to be detected, wherein the spectral patterns of sequential expression of nucleic

acids and proteins in normal skin tissue are used as a baseline for the comparison; and

(vii) comparing the expression of nucleic acids and proteins in detected peaks values and their intensities obtained on a biopsied tissue sample to identify differences corresponding to specific expression of nucleic acids and proteins with carcinogenesis of the tissue to be detected in certain skin tumour types, wherein normal skin tissue numerical ranges of mean peaks values and intensities for nucleic acids and proteins are used as references for the comparison.

2. The method of Claim 1, further comprising the analysis of a systemic progression of the grade of activity of expressed nucleic acids and proteins contributing to carcinogenesis from benign to malignant skin tumourous tissue.

3. The method of any of Claims 1-2, wherein the spectral patterns of sequentially expressed nucleic acids and proteins in FTIR spectra for benign skin tumourous tissues with reference to BCN, is further characterized by the following features:

1 BCN spectral pattern of sequential expression of nucleic acids, based on mean peaks values and intensities

as *(965 cm^{-1}) < (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* versus [0.7:1], or [0.8:1], or [0.9:1],

wherein

BCN spectral pattern of nucleic acids as *(965 cm^{-1}) < (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* is characterized by

mean DNA peak values at 965 cm^{-1} within 0.12-0.18,

mean DNA/RNA peak values at 1055 cm^{-1} within 0.17-0.24,

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.19-0.27,

mean DNA peak intensities at 965 cm^{-1} within 0.47-0.56,

mean DNA/RNA peak intensities at 1055 cm^{-1} within 0.59-0.75,

mean DNA/RNA triad max peak intensities at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.72-0.84; and

1 among 3 BCN spectral patterns of sequential expression of proteins, based on mean peaks values and intensities

as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus [0.7:1], or

as *(1245 cm^{-1}) > (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus [0.7:1], or [0.8:1], or

as *(1245 cm^{-1}) > (1310 cm^{-1}) < (1390 cm^{-1}) < (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus [0.9:1],

wherein

BCN spectral pattern of proteins as *(1245 cm^{-1}) > (1310 cm^{-1}) < (1390 cm^{-1}) < (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* is characterized by mean peaks values and intensities within (0.3) > (0.2) < (0.3) < (0.4) < (0.7) < (1.0),

BCN spectral pattern of proteins as *(1245 cm^{-1}) > (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* is characterized by mean peaks values and intensities within (0.3) > (0.2) < (0.3) = (0.3) < (0.7) < (1.0),

BCN spectral pattern of proteins as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* is characterized by mean peaks values and intensities within (0.3) = (0.3) < (0.4) = (0.4) < (0.8) < (1.0).

4. The method of any of Claims 1-2, wherein the spectral patterns of sequentially expressed nucleic acids and proteins in FTIR spectra for premalignant skin tumourous tissues with reference to BD, is further characterized by the following features:

1 among 2 BD spectral patterns of sequential expression of nucleic acids, based on mean peaks values and intensities

as *(965 cm^{-1}) < (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* versus [0.8:1], or [0.9:1], or

as *(no peak expression) (no peak expression) (1071, 1084/1085, 1095 cm^{-1})* versus [0.7:1],

wherein

BD spectral pattern of nucleic acids as *(965 cm^{-1}) < (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* is characterized by

mean DNA peak values at 965 cm^{-1} within 0.09-0.23,

mean DNA/RNA peak values at 1055 cm^{-1} within 0.15-0.26,

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.18-0.29,

mean DNA peak intensities at 965 cm^{-1} within 0.41-0.70,

mean DNA/RNA peak intensities at 1055 cm^{-1} within 0.68-0.79,

mean DNA/RNA triad max peak intensities at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.82-0.88,

BD spectral pattern of nucleic acids as *(no peak expression) (no peak expression) (1071, 1084/1085, 1095 cm^{-1})* is characterized by

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.07,

mean DNA/RNA triad max peak intensities at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.64; and

1 BD spectral pattern of sequential expression of proteins, based on mean peaks values and intensities

as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus [0.7:1], or [0.8:1], or [0.9:1],

wherein

BD spectral pattern of proteins as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* is characterized by mean peaks values and intensities within

(0.3) = (0.3) < (0.4) = (0.4) < (0.8) < (1.0), or

(0.2) = (0.2) < (0.3) = (0.3) < (0.7) < (1.0), or

(0.1) = (0.1) < (0.2) = (0.2) < (0.6) < (1.0).

5. The method of any of Claims 1-2, wherein the spectral patterns of sequentially expressed nucleic acids and proteins in FTIR spectra for malignant skin tumourous tissues with reference to MM, is further characterized by the following features:

1 among 3 MM spectral patterns of sequentially expressed nucleic acids, based on mean peaks values and intensities

as (*965 cm^{-1}) < (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* versus [0.8:1], or

as *(965 cm^{-1}) > (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* versus [0.8:1], or

as *(965 cm^{-1}) > (no peak expression) < (1071, 1084/1085, 1095 cm^{-1})* versus [0.7:1],

wherein

MM spectral pattern of nucleic acids as (*965 cm^{-1}) < (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* is characterized by

mean DNA peak values at 965 cm^{-1} within 0.23,

mean DNA/RNA peak values at 1055 cm^{-1} within 0.26,

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.30,

mean DNA peak intensities at 965 cm^{-1} within 0.61,

mean DNA/RNA peak intensities at 1055 cm^{-1} within 0.69,

mean DNA/RNA triad max peak intensities at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095

cm^{-1} within 0.79,

MM spectral pattern as *(965 cm^{-1}) > (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* is charactrized by

mean DNA peak values at 965 cm^{-1} within 0.19,

mean DNA/RNA peak values at 1055 cm^{-1} within 0.14,

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.27,

mean DNA peak intensities at 965 cm^{-1} within 0.63,

mean DNA/RNA peak intensities at 1055 cm^{-1} within 0.47,

mean DNA/RNA triad max peak intensities at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.90,

MM spectral pattern as *(965 cm^{-1}) > (no peak expression) < (1071, 1084/1085, 1095 cm^{-1})* is charactrized by

mean DNA peak values at 965 cm^{-1} within 0.25,

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.35,

mean DNA peak intensities at 965 cm^{-1} within 0.52,

mean DNA/RNA triad max peak intensities at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.73; and

1 among 2 MM spectral patterns of sequentially expressed proteins, based on mean peaks values and intensities

as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus [0.8:1], or

as *(1245 cm^{-1}) > (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus [0.7:1],

wherein

MM spectral pattern of proteins as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* is characterized by mean peaks values and intensities within

(0.3) = (0.3) < (0.4) = (0.4) < (0.8) < (1.0), or

(0.4) = (0.4) < (0.5) = (0.5) < (0.8) < (1.0),

MM spectral pattern of proteins as *(1245 cm^{-1}) > (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* is characterized by mean peaks values and intensities within (0.5) > (0.4) < (0.5) = (0.5) < (0.8) < (1.0).

6. The method of any of Claims 1-2, wherein the spectral patterns of sequentially expressed nucleic acids and proteins in FTIR spectra for malignant skin tumourous tissues with reference to SCC, is further characterized by the following features:

1 among 4 SCC spectral patterns of sequentially expressed nucleic acids, based on mean peaks values and intensities

as *(965 cm^{-1}) > (no peak expression) < (1071, 1084/1085, 1095 cm^{-1})* versus [0.7:1], or

as *(965 cm^{-1}) > (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* versus [0.7:1], or

as *(965 cm^{-1}) < (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* versus [0.9:1], or

as *(965 cm^{-1}) < (1055 cm^{-1}) > (1071, 1084/1085, 1095 cm^{-1})* versus [1:1],

wherein

SCC spectral pattern of nucleic acids as *(965 cm^{-1}) > (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* is charactrized by

mean DNA peak values at 965 cm^{-1} within 0.19,

mean DNA/RNA peak values at 1055 cm^{-1} within 0.13,

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.16,

SCC spectral pattern of nucleic acids as *(965 cm^{-1}) < (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* is charactrized by

mean DNA peak values at 965 cm^{-1} within 0.26,

mean DNA/RNA peak values at 1055 cm^{-1} within 0.30,

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.35,

mean DNA peak intensities at 965 cm^{-1} within 0.70,

mean DNA/RNA peak intensities at 1055 cm^{-1} within 0.81,

mean DNA/RNA triad max peak intensities at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.88,

SCC spectral pattern of nucleic acids as *(965 cm^{-1}) < (1055 cm^{-1}) > (1071, 1084/1085, 1095 cm^{-1})* is characterized by

mean DNA peak values at 965 cm^{-1} within 0.60,

mean DNA/RNA peak values at 1055 cm^{-1} within 0.66,

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.65,

mean DNA peak intensities at 965 cm^{-1} within 0.41, or 0.91,

mean DNA/RNA peak intensities at 1055 cm^{-1} within 0.77, or 1.00,

mean DNA/RNA triad max peak intensities at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.71, or 0.99,

SCC spectral pattern of nucleic acids *(965 cm^{-1}) > (no peak expression) < (1071, 1084/1085, 1095 cm^{-1})* is charactrized by

mean DNA peak values at 965 cm^{-1} within 0.07,

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.12,

mean DNA peak intensities at 965 cm^{-1} within 0.91,

mean DNA/RNA triad max peak intensities at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.76; and

1 among 3 SCC spectral patterns of sequentially expressed proteins, based on mean peaks values and intensities

as *(1245 cm^{-1}) = (1310 cm^{-1}) = (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus [0.7:1], or [0.9:1], or

as *(1245 cm^{-1}) > (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus [1:1], or

as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus [0.7:1],

wherein

SCC spectral pattern of proteins as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* is characterized by mean peaks values and intensities within

(0.2) = (0.2) = (0.2) = (0.2) < (0.6) < (1.0), or

(0.4) = (0.4) = (0.4) = (0.4) < (0.7) < (1.0),

SCC spectral pattern of proteins as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* is characterized by mean peaks values and intensities within

(0.2) = (0.2) < (0.3) = (0.3) < (0.6) < (1.0),

SCC spectral pattern of proteins as *(1245 cm^{-1}) > (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* is characterized by mean proteins peaks values and intensities within

(0.7) = (0.6) < (0.7) = (0.7) < (0.9) < (1.0).

7. The method of any of Claims 1-2, wherein the spectral patterns of sequentially expressed nucleic acids and proteins in FTIR spectra for malignant skin tumourous tissues associated with BCC, is further characterized by the following features:

1 among 3 BCC spectral patterns of sequentially expressed nucleic acids, based on mean peaks values and intensities

as *(965 cm^{-1}) = (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* versus [0.8:1], or

as *(965 cm^{-1}) < (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* versus [0.8:1], or [1:1], or

as *(965 cm^{-1}) < (1055 cm^{-1}) > (1071, 1084/1085, 1095 cm^{-1})* versus [0.8:1], or [1:1], wherein

BCC spectral pattern of nucleic acids as *(965 cm^{-1}) = (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* is characterized by

mean DNA peak values at 965 cm^{-1} within 0.09,

mean DNA/RNA peak values at 1055 cm^{-1} within 0.09,

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.23,

mean DNA peak intensities at 965 cm^{-1} within 0.45,

mean DNA/RNA peak intensities at 1055 cm^{-1} within 0.45,

mean DNA/RNA triad max peak intensities at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 1.15,

BCC spectral pattern of nucleic acids as *(965 cm^{-1}) < (1055 cm^{-1}) < (1071, 1084/1085, 1095 cm^{-1})* is characterized by

mean DNA peak values at 965 cm^{-1} within 0.08, or 0.31, or 0.35,

mean DNA/RNA peak values at 1055 cm^{-1} within 0.14, or 0.43, or 0.44,

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.16, or 0.45, or 0.48,

mean DNA peak intensities at 965 cm^{-1} within 0.38, or 0.71,

mean DNA/RNA peak intensities at 1055 cm^{-1} within 0.67, or 0.98,

mean DNA/RNA triad max peak intensities at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.76, or 1.09,

BCC spectral pattern of nucleic acids as *(965 cm^{-1}) < (1055 cm^{-1}) > (1071, 1084/1085, 1095 cm^{-1})* is charactrized by

mean DNA peak values at 965 cm^{-1} within 0.11, or 0.35,

mean DNA/RNA peak values at 1055 cm^{-1} within 0.19, or 0.42,

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.18, or 0.40,

mean DNA peak intensities at 965 cm^{-1} within 0.50, or 0.88,

mean DNA/RNA peak intensities at 1055 cm^{-1} within 0.87, or 1.05,

mean DNA/RNA triad max peak intensities at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.82, or 1.00; and

1 among 2 BCC spectral patterns of sequentially expressed proteins, based on mean peaks values and intensities

as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus [0.8:1], or [1:1], or

as *(1245 cm^{-1}) > (1310 cm^{-1}) = (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus [1:1],

wherein

BCC spectral pattern of proteins as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* is characterized by mean peaks values and intensities within

(0.2) = (0.2) < (0.3) = (0.3) < (0.7) < (1.0), or

(0.2) = (0.2) < (0.3) = (0.3) < (0.8) < (1.0), or

(0.4) = (0.4) < (0.5) = (0.5) < (0.8) < (1.0),

BCC spectral pattern of proteins as *(1245 cm^{-1}) > (1310 cm^{-1}) = (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* is characterized by mean peaks values and intensities within (0.5) > (0.4) = (0.4) = (0.4) < (0.8) < (1.0).

8. The method of any of Claims 1-2, wherein the spectral patterns of sequentially expressed nucleic acids and proteins in FTIR spectra for normal skin tissue with reference to HS epidermis, is further characterized by the following features:

1 HS epidermis spectral pattern of sequentially expressed nucleic acids, based on

mean peaks values and intensities

as *(965 cm^{-1}) > (no peak expression) < (1071, 1084/1085, 1095 cm^{-1})* versus [0.5:1], or [0.7:1],

wherein

mean DNA peak values at 965 cm^{-1} within 0.15-0.24,

mean DNA/RNA triad max peak values at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.22-0.47,

mean DNA peak intensities at 965 cm^{-1} within 0.38-0.51,

mean DNA/RNA triad max peak intensities at 1071 cm^{-1}, 1084/1085 cm^{-1} or 1095 cm^{-1} within 0.49-1.00.

9. The method of any of Claims 1-2, further comprising one of the following:

comparing any received spectral patterns of sequentially expressed nucleic acids and proteins with referenced spectral patterns of sequentially expressed nucleic acids and proteins to be specific for benign, premalignant or malignant skin tumourous tissue with reference to BCC; SCC; MM; BD or BCN, in comparison to normal skin tissue; or

determining in any received spectral patterns of sequentially expressed nucleic acids and proteins which of the differences contribute to the carcinogenesis to be detected between normal and premalignant skin tissue cells, or between normal and malignant skin tissue cells, or between premalignant and malignant skin tissue cells; or

further analysing any received spectral patterns of sequentially expressed nucleic acids and proteins for a systemic progression of the grade of activity of nucleic acids and proteins between normal and benign skin tumourous tissues, characteristic for tumour progression towards benign skin tumourous tissue with reference to BCN; or

further analysing any received spectral patterns of sequentially expressed nucleic acids and proteins for a systemic progression of the grade of activity of nucleic acids and proteins between normal and premalignant skin tumourous tissues, characteristic for tumour progression towards premalignant skin tumourous tissue with reference to BD; or

further analysing any received spectral patterns of sequentially expressed nucleic acids and proteins for a systemic progression of the grade of activity of nucleic acids and proteins between normal and malignant skin tumourous tissues, characteristic for tumour progression towards malignant skin tumourous tissue with reference to MM, or with BCC, or with reference to SCC; or

further analysing received spectral patterns of sequentially expressed nucleic acids and proteins for a systemic progression of the grade of activity of nucleic acids and proteins within one type of tumour in association with MM to be characteristic for

malignant skin tumour progression in the same patient, wherein previously received spectral patterns of sequentially expressed nucleic acids and proteins were characteristic for benign skin tumourous tissue with reference to BCN, in comparison to normal skin tissue; or

further analysing received spectral patterns of sequentially expressed nucleic acids and proteins for a systemic progression of the grade of activity of nucleic acids and proteins within one type of tumour with reference to SCC to be characteristic for malignant skin tumour progression in the same patient, wherein previously received spectral patterns of sequentially expressed nucleic acids and proteins were characteristic for premalignant tumourous skin tissue with reference to BD, in comparison to normal skin tissue.

10. The method of Claims 1-9, wherein said expression levels of nucleic acids and proteins peaks are determined in normalized FTIR spectra to amide I at 1650 cm^{-1}.

11. The method of Claims 1-9, wherein DNA/RNA triad peak (1071, 1084/1085, 1095 cm^{-1}) is defined by the maximum peak in a triad.

12. The method of Claims 1-9, wherein the numerical values of peaks values of expressed proteins in FTIR spectra are numerically equalled to numercial values of calculated intensities of corresponding protein peaks in FTIR spectra normalized to amide I at 1650 cm^{-1}.