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 carcinogenesis in 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 in patterns 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 about 965 cm^{-1}; the multiplet (DNA/RNA) at about 1055 cm^{-1}; DNA/RNA triad peaks at about 1071 cm^{-1}, 1084/1085 cm^{-1}, 1095 cm-1; at about 1245 cm^{-1} as a combination for DNA and amide III; a number of non-specific (non-descriptive) proteins at about 1310 cm^{-1}, 1390 cm^{-1} and 1450 cm^{-1}; the amide II vibration at about 1540 cm^{-1}; and the amide I vibration at about 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 sequentially expressed in patterns in infrared (IR) spectra of benign skin tumours, epidermal skin precancers and cancers 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 carcinogenesis in human biopsied tumourous tissue samples by means of spectral patterns analysis of sequentially expressed nucleic acids and proteins in those. The sample can be comprised of normal, benign, precancerous or cancerous 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 in spectral sequential patterns for each type of cancer and between different types of cancers, 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 cancer generally develops in the epidermis. Most common malignant skin cancers are malignant skin cancers are basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma (MM), that have been used as examples of malignant neoplastic skin disease 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 precancerous skin lesion, well-known as intraepidermal squamous cell carcinoma *in situ*. Benign compound nevus 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 and 1300 cm-1 for nucleic acids and the specified wavenumber region between 1300 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 CaF2 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 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 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 tumours: basal cell carcinoma (BCC) (6 patients), squamous cell carcinoma (SCC) (4 patients), malignant melanoma (MM) (3 patients); from 3 patients with precancers: Bowen's disease (3 patients); from 4 patients with benign tumours: benign compound nevus (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 spectral patterns in the IR spectra of BCC, SCC, MM, Bowen's disease and benign compound nevus, in comparison to IR spectra of normal skin from healthy subjects:

- determining spectral sequential pattern of nucleic acids from assigned in the wavenumber region of 900-1300 cm^{-1} spectral peaks to nucleic acids at 965 cm^{-1},

1055 cm^{-1}, 1071 cm^{-1}, 1084/1085 cm^{-1}, 1095 cm^{-1}

- determining spectral sequential pattern of nucleic acids from detected mean values of the expression level of each nucleic acid peak
- determining spectral sequential pattern of nucleic acids from calculated intensities of each assigned nucleic acid peak to the intensity of the peak at about 1245 cm^{-1} in the following ratios: I(965)/I(1245), I(1055)/I(1245), I(max peak in DNA/RNA triad peaks)/I(1245)
- determining spectral sequential pattern of proteins from assigned in the wavenumber region of 1300-1700 cm^{-1} spectral peaks to specific and non-specific proteins at about 1245 cm^{-1}, 1310 cm^{-1}, 1390 cm^{-1}, 1450 cm^{-1}, 1540 cm^{-1}, 1650 cm^{-1}
- determining spectral sequential pattern of proteins from calculated mean values of expression levels of each assigned (specific and non-specific) protein peak
- determining spectral sequential pattern of proteins from calculated intensities of each assigned peak for specific and nonspecific proteins to the intensity of amide I peak at about 1650 cm^{-1} in the following ratios: I(1310)/I(1650), I(1390)/I(1650), I(1450)/I(1650), I(1540)/I(1650)
- calculating the level of activity of DNA-RNA versus DNA-protein interactions by proportional ratio: I(sum mean DNA/RNA triad peaks)/I(1245)
- comparing the level of activity of multiplet (DNA/RNA) with the level(s) of activity of the peaks in DNA/RNA triad in received sequential patterns of nucleic acids and proteins of benign, premalignant and malignant skin tumours in correlation to calculated proportional ratios

Below is a summary of the data employed from the patients with BCC, SCC, MM, Bowen's disease, benign compound nevus and healthy subjects, provided in examples.

INTRA- AND INTER-MOLECULAR INTERACTIONS FOR NUCLEIC ACIDS IN PATTERNS

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}).

3 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]

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) < I(1055)/I(1245) < 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}).

2 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*

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]

2 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)*

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}).

1 BD spectral pattern of nucleic acids in 3 patients, based on expressed mean values:

- *965 < 1055 < 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]

1 BD spectral pattern of nucleic acids in 3 patients, based on the intensity ratios:

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

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 traid 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 about 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, being higher in malignant skin tumours.

Pattern recognition on DNA-RNA and DNA-DNA interactions as I*965* < I*1055* < I *max level DNA/RNA triad 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 Bowen's disease and benign compound nevus, again 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 *max level DNA/RNA triad peak* was clearly seen in 3 SCC patients with the highest activity levels of the multiplet at about 1055 cm^{-1}.

In 2 patients with MM pattern recognition on DNA-RNA and DNA-DNA interactions as I*965* > I*1055* < I *max level DNA/RNA triad 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* > *(no peak expression)* < I *max level DNA/RNA triad peak* was different from all measured above pathologies, with no expression of the multiplet at about 1055 cm^{-1} in

the epidermis.

INTRA- AND INTER-MOLECULAR INTERACTIONS FOR PROTEINS IN PATTERNS

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}).
In normalized spectra to amide I at about 1650 cm-1 numerical values detected from the expression levels of proteins peaks are numerically equaled to calculated intensities of proteins peaks in IR spectra.

EXAMPLE 1
BCC

2 BBC 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) 0.5 > 0.4 = 0.4 = 0.4 < 0.7 < 1.0 vs. [1:1]
2) 0.5 > 0.4 = 0.4 = 0.4 < 0.7 < 1.0 vs. [1:1]
3) 0.4 = 0.4 < 0.5 = 0.5 < 0.8 < 1.0 vs. [1:1]
4) 0.2 = 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.8:1]
5) 0.2 = 0.2 < 0.3 = 0.3 < 0.8 < 1.0 vs. [0.8:1]
6) 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) 0.7 > 0.6 < 0.7 = 0.7 < 0.9 < 1.0 vs. [1:1]
2) 0.4 = 0.4 = 0.4 = 0.4 < 0.7 < 1.0 vs. [0.9:1]
3) 0.2 = 0.2 < 0.3 = 0.3 < 0.6 < 1.0 vs. [0.7:1]
4) 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) 0.4 = 0.4 < 0.5 = 0.5 < 0.8 < 1.0 vs. [0.8:1]
2) 0.3 = 0.3 < 0.4 = 0.4 < 0.8 < 1.0 vs. [0.8:1]
3) 0.5 > 0.4 < 0.5 = 0.5 < 0.8 < 1.0 vs. [0.7:1]

EXAMPLE 4
BOWEN's DISEASE (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

1) 0.3 = 0.3 < 0.4 = 0.4 < 0.8 < 1.0 vs. [0.9:1]
2) 0.2 = 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.8:1]
3) 0.1 = 0.1 < 0.2 = 0.2 < 0.6 < 1.0 vs. [0.7:1]

EXAMPLE 5

BENIGN COMPOUND NEVUS (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

1) 0.3 > 0.2 < 0.3 < 0.4 < 0.7 < 1.0 vs. [0.9:1]
2) 0.3 > 0.2 < 0.3 < 0.4 < 0.7 < 1.0 vs. [0.9:1]
3) 0.3 > 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.8:1]
4) 0.3 = 0.3 < 0.4 = 0.4 < 0.8 < 1.0 vs. [0.7:1]

EXAMPLE 6
HEALTHY SKIN (HS)

1 HS spectral pattern of proteins from 4 healthy subjects with normal skin, based on expressed mean values and/or calculated intensity ratios:

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

1) 0.5 > 0.3 < 0.5 < 0.8 < 1.0 vs. [0.5:1]
2) 0.5 > 0.3 < 0.5 < 0.8 < 1.0 vs. [0.5:1]
3) 0.6 > 0.4 < 0.5 < 0.8 < 1.0 vs. [0.7:1]
4) 0.6 > 0.4 < 0.5 < 0.8 < 1.0 vs. [0.7:1]

Example 7
MM vs. BENIGN COMPOUND NEVUS (BCN)

2 MM spectral patterns of proteins versus 2 BCN spectral patterns of proteins:

MM: 1245 = 1310 < 1390 = 1450 < 1540 < 1650 vs. [0.8:1]
MM: 1245 > 1310 < 1390 = 1450 < 1540 < 1650 vs. [0.7:1]
BCN: 1245 = 1310 < 1390 = 1450 < 1540 < 1650 vs. [0.7:1]
BCN: 1245 > 1310 < 1390 < 1450 < 1540 < 1650 vs. [0.8:1] - [0.9:1]

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 < 0.4 < 0.7 < 1.0 vs. [0.9:1]
3) BCN: 0.3 > 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.8:1]
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 8
SCC vs. BOWEN's DISEASE (BD)

1 BD spectral pattern of proteins versus 3 SCC spectral patterns of proteins:

BD: 1245 = 1310 < 1390 = 1450 < 1540 < 1650 vs. [0.7:1]-[0.9:1]
SCC: 1245 = 1310 < 1390 = 1450 < 1540 < 1650 vs. [0.7:1]-[0.9:1]
SCC: 1245 = 1310 = 1390 = 1450 < 1540 < 1650 vs. [0.7:1], [0.9:1]
SCC: 1245 > 1310 < 1390 = 1450 < 1540 < 1650 vs. [1:1]

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 9
ALL MEASURED PATHOLOGIES

3 SCC spectral patterns versus 2 BBC spectral patterns versus 2 MM spectral patterns 1 BD spectral pattern versus 2 BCN spectral patterns versus 1 HS spectral pattern of proteins:

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) BCN: 0.3 > 0.2 < 0.3 = 0.3 < 0.7 < 1.0 vs. [0.8:1]
9) MM: 0.4= 0.4 < 0.5 = 0.5 < 0.8 < 1.0 vs. [0.8:1]
10) MM: 0.3 = 0.3 < 0.4 = 0.4 < 0.8 < 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 < 0.7 < 1.0 vs. [0.8:1]
13) BCC: 0.2 = 0.2 < 0.3 = 0.3 <0.7 < 1.0 vs. [0.8:1]
14) BD: 0.2 = 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 = 0.3 < 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, 1390 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 about 1310, 1390 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 sequenced pattern analysis of expressed nucleic acids and proteins in FTIR spectra for characterization of a multi-stage human skin carcinogenesis in a skin tissue sample, comprising of:

(i) recognizing patterned sequences of nucleic acids from determined precise order of expressed nucleic acid peaks corresponding to common expression of the nucleic acid with carcinogenesis of the tissue to be detected in certain skin tumour type, wherein
a sequence DNA (965 cm^{-1}), DNA/RNA triad (1071,1084/1085,1095 cm^{-1}) is associated with normal tissue, and
a sequence DNA (965 cm^{-1}), DNA/RNA (1055 cm^{-1}), DNA/RNA triad (1071,1084/1085,1095 cm^{-1}) is associated with benign, premalignant and malignant skin tumourous tissue;

(ii) recognizing patterned sequences of nucleic acids from detected expressed nucleic acids peak values and their calculated intensities corresponding to the specific expression of the nucleic acid with carcinogenesis of the tissue to be detected in certain skin tumour type, wherein nucleic acid sequence patterns for

normal tissue is (965) < (1071,1084/1085,1095),
benign tissue is (965) < (1055) < (1071,1084/1085,1095),
premalignant tissue is (965) < (1055) < (1071,1084/1085,1095),
malignant tissue is
(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
[0.5:1] - [0.7:1] is for normal tissue,
[0.7:1] - [0.9:1] is for benign tissue,
[0.8:1] - [0.9:1] is for premalignant tissue,

[0.7:1] - [1:1] is for malignant tissue;

(iv) recognizing patterned sequences of proteins from detected peak values and their calculated intensities of expressed proteins corresponding to the specific expression of the proteins with carcinogenesis of the tissue to be detected in certain skin tumour type, wherein protein sequence patterns for

normal tissue is (1310) > (1390) < (1450) < (1540) < (1650),
benign tissue is
(1310) < (1390) < (1450) < (1540) < (1650) or
(1310) < (1390) = (1450) < (1540) < (1650),
premalignant tissue is
(1310) < (1390) = (1450) < (1540) < (1650),
malignant tissue is
(1310) < (1390) < (1450) < (1540) < (1650) or
(1310) = (1390) = (1450) < (1540) < (1650);

(v) correlating said patterned sequences of nucleic acids and proteins with said proportional ratio to characterize the expression of nucleic acids and proteins with carcinogenesis of the tissue to be detected in certain skin tumour type;

(vi) comparing the expression of nucleic acids and proteins in recognized sequences obtained in a skin tissue sample to identify differences corresponding to the specific expression of nucleic acids and proteins with carcinogenesis of the tissue to be detected in certain skin tumour type, wherein normal tissue nucleic acid and protein patterned sequences are used as a baseline for the comparison; and

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

2. The method of Claim 1, wherein determining the spectral sequential patterns of

nucleic acids and proteins, specific for benign tumours of skin tissue associated with benign compound nevus, specific for premalignant tumours of skin tissue associated with Bowen's disease, specific for malignant tumours of skin tissue associated with basal cell carcinoma, specific for malignant tumours of skin tissue associated with squamous cell carcinoma, specific for malignant tumours of skin tissue associated with malignant melanoma.

3. 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.

4. The method of any of Claims 1-3, in which said benign skin tumourous tissue associated with benign compound nevus is further characterized by the following features:

1 spectral pattern of sequentially expressed mean peak values and/or intensities of nucleic acids as *(965 cm^{-1}) < (1055 cm^{-1}) < (1071,1084/1085,1095 cm^{-1})* versus to any ratio between [0.7:1] and [0.9:1];

the numerical ranges of mean values of DNA peak at 965 cm^{-1} within 0.12-0.18;

the numerical ranges of mean values of DNA/RNA peak at 1055 cm^{-1} within 0.17-0.24;

the numerical ranges of mean values of the maximum peak in DNA/RNA triad within 0.19-0.27;

the numerical ranges of calculated intensities of DNA peak at 965 cm^{-1} within 0.48-0.56;

the numerical ranges of calculated intensities of DNA/RNA peak at 1055 cm^{-1} within 0.68-0.75;

the numerical ranges of calculated intensities of the maximum peak in DNA/RNA triad within 0.76-0.84;

3 spectral patterns of sequentially expressed mean peak values and/or intensities of proteins as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus to [0.7:1], or as *(1245 cm^{-1}) > (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus to [0.8:1], or as *(1245 cm^{-1}) > (1310 cm^{-1}) < (1390 cm^{-1}) < (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus to [0.9:1];

the numerical ranges of mean values of protein peak at 1310 cm^{-1} within 0.2-0.3;

the numerical ranges of mean values of protein peak at 1390 cm^{-1} within 0.3-0.4;

the numerical ranges of mean values of protein peak at 1450 cm^{-1} within 0.3-0.4;

the numerical range of mean values of protein peak at 1540 cm^{-1} within 0.7-0.8; and/or characteristic proportional ratio within [0.7:1] – [0.9:1] to be dependent on low grade of activity level of expressed nucleic acids and proteins in spectral patterns of nucleic acids and proteins, corresponding to common and specific expression of the nucleic acids and proteins with carcinogenesis of benign skin tissue to be detected.

5. The method of any of Claims 1-3, in which said premalignant skin tumourous tissue associated with Bowen's disease is further characterized by the following features:
1 spectral pattern of sequentially expressed mean peak values and/or intensities of nucleic acids as *(965 cm^{-1}) < (1055 cm^{-1}) < (1071,1084/1085,1095 cm^{-1})* versus to [0.8:1] or [0.9:1];
the numerical ranges of mean values of DNA peak at 965 cm^{-1} within 0.09-0.23;
the numerical ranges of mean values of DNA/RNA peak at 1055 cm^{-1} within 0.15-0.26;
the numerical ranges of mean values of the maximum peak in DNA/RNA triad within 0.18-0.29;
the numerical ranges of calculated intensities of DNA peak at 965 cm^{-1} within 0.41-0.70;
the numerical ranges of calculated intensities of DNA/RNA peak at 1055 cm^{-1} within 0.68-0.79;
the numerical ranges of calculated intensities of the maximum peak in DNA/RNA triad within 0.82-0.88;
1 spectral pattern of sequentially expressed mean peak values and/or intensities of proteins as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus to [0.8:1] or [0.9:1];
the numerical ranges of mean values of protein peak at 1310 cm^{-1} within 0.1-0.3;
the numerical ranges of mean values of protein peak at 1390 cm^{-1} within 0.2-0.4;
the numerical ranges of mean values of protein peak at 1450 cm^{-1} within 0.2-0.4;
the numerical range of mean values of protein peak at 1540 cm^{-1} within 0.6-0.8; and/or characteristic proportional ratio within [0.8:1] — [0.9:1] to be dependent on medium grade of activity level of expressed nucleic acids and proteins in spectral patterns of nucleic acids and proteins, corresponding to common and specific expression of the nucleic acids and proteins with carcinogenesis of precancerous skin tissue to be detected.

6. The method of any of Claims 1-3, in which said malignant skin tumourous tissue

associated with malignant melanoma is further characterized by the following features:
2 spectral patterns of sequentially expressed mean peak values and/or intensities of nucleic acids as (*965 cm^{-1}) < (1055 cm^{-1}) < (1071,1084/1085,1095 cm^{-1})* versus to [0.8:1], or as *(965 cm^{-1}) > (1055 cm^{-1}) < (1071,1084/1085,1095 cm^{-1})* versus to [0.8:1];
the numerical ranges of mean values of DNA peak at 965 cm^{-1} within 0.19-0.25;
the numerical ranges of mean values of DNA/RNA peak at 1055 cm^{-1} within 0.14-0.26;
the numerical ranges of mean values of the maximum peak in DNA/RNA triad within 0.27-0.35;
the numerical ranges of calculated intensities of DNA peak at 965 cm^{-1} within 0.52-0.63;
the numerical ranges of calculated intensities of DNA/RNA peak at 1055 cm^{-1} within 0.47-0.69;
the numerical ranges of calculated intensities of the maximum peak in DNA/RNA triad within 0.73-0.90;
2 spectral patterns of sequentially expressed mean peak values and/or intensities of proteins as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus to [0.8:1], or as *(1245 cm^{-1}) > (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus to [0.8:1];
the numerical ranges of mean values of protein peak at 1310 cm^{-1} within 0.3-0.4;
the numerical ranges of mean values of protein peak at 1390 cm^{-1} within 0.4-0.5;
the numerical ranges of mean values of protein peak at 1450 cm^{-1} within 0.4-0.5;
the numerical range of mean values of protein peak at 1540 cm^{-1} at 0.8; and/or
characteristic proportional ratio within [0.7:1] — [0.8:1] to be dependent on medium grade of activity level of expressed nucleic acids and proteins in sequential patterns of nucleic acids and proteins, corresponding to common and specific expression of the nucleic acids and proteins with carcinogenesis of cancerous skin tissue to be detected.

7. The method of any of Claims 1-3 , in which said malignant skin tumourous tissue associated with squamous cell carcinoma is further characterized by the following features:
3 spectral patterns of sequentially expressed mean peak values and/or intensities of nucleic acids as *(965 cm^{-1}) > (1055 cm^{-1}) < (1071,1084/1085,1095 cm^{-1})* versus to [0.7:1], or as *(965 cm^{-1}) < (1055 cm^{-1}) < (1071,1084/1085,1095 cm^{-1})* versus to [0.9:1], or as *(965 cm^{-1}) < (1055 cm^{-1}) > (1071,1084/1085,1095 cm^{-1})* versus to [1:1];
the numerical ranges of mean values of DNA peak at 965 cm^{-1} within 0.07-0.60;

the numerical ranges of mean values of DNA/RNA peak at 1055 cm^{-1} within 0.13-0.66;
the numerical ranges of mean values of the maximum peak in DNA/RNA triad within 0.12-0.65;
the numerical ranges of calculated intensities of DNA peak at 965 cm^{-1} within 0.41-0.91;
the numerical ranges of calculated intensities of DNA/RNA peak at 1055 cm^{-1} within 0.77-1.00;
the numerical ranges of calculated intensities of the maximum peak in DNA/RNA triad within 0.71-0.99;
3 spectral patterns of sequentially expressed mean peak values and/or intensities of proteins as *(1245 cm^{-1}) = (1310 cm^{-1}) = (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus to [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 to [1:1], or as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus to [0.7:1];
the numerical ranges of mean values of protein peak at 1310 cm^{-1} within 0.2-0.6;
the numerical ranges of mean values of protein peak at 1390 cm^{-1} within 0.2-0.7;
the numerical ranges of mean values of protein peak at 1450 cm^{-1} within 0.2-0.7;
the numerical range of mean values of protein peak at 1540 cm^{-1} within 0.6-0.9; and/or characteristic proportional ratio within [0.7:1] — [1:1] to be dependent on low, medium or high grade of activity level of expressed nucleic acids and proteins in sequential patterns of nucleic acids and proteins, corresponding to common and specific expression of the nucleic acids and proteins with carcinogenesis of cancerous skin tissue to be detected.

8. The method of any of Claims 1-3, in which said malignant skin tumourous tissue associated with basal cell carcinoma is further characterized by the following features:
3 spectral patterns of sequentially expressed mean peak values and/or intensities of nucleic acids as *(965 cm^{-1}) = (1055 cm^{-1}) < (1071,1084/1085,1095 cm^{-1})* versus to [0.8:1], or as *(965 cm^{-1}) < (1055 cm^{-1}) < (1071,1084/1085,1095 cm^{-1})* versus to [0.8:1] or [1:1], or as *(965 cm^{-1}) < (1055 cm^{-1}) > (1071,1084/1085,1095 cm^{-1})* versus to [0.8:1] or [1:1];
the numerical ranges of mean values of DNA peak at 965 cm^{-1} within 0.09-0.35;
the numerical ranges of mean values of DNA/RNA peak at 1055 cm^{-1} within 0.09-0.44;
the numerical ranges of mean values of the maximum peak in DNA/RNA triad within 0.16-0.48;

the numerical ranges of calculated intensities of DNA peak at 965 cm^{-1} within 0.38-0.88;
the numerical ranges of calculated intensities of DNA/RNA peak at 1055 cm^{-1} within 0.45-1.05;
the numerical ranges of calculated intensities of the maximum peak in DNA/RNA triad within 0.76-1.15;
2 spectral patterns of sequentially expressed mean peak values and/or intensities of proteins as *(1245 cm^{-1}) = (1310 cm^{-1}) < (1390 cm^{-1}) = (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus to [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 to [1:1];
the numerical ranges of mean values of protein peak at 1310 cm^{-1} within 0.2-0.4;
the numerical ranges of mean values of protein peak at 1390 cm^{-1} within 0.3-0.5;
the numerical ranges of mean values of protein peak at 1450 cm^{-1} within 0.3-0.5;
the numerical range of mean values of protein peak at 1540 cm^{-1} within 0.7-0.8; and/or characteristic proportional ratio within [0.8:1] — [1:1] to be dependent on low, medium or high grade of activity level of expressed nucleic acids and proteins in sequential patterns of nucleic acids and proteins, corresponding to common and specific expression of the nucleic acids and proteins with carcinogenesis of cancerous skin tissue to be detected.

9. The method of any of Claims 1-3, in which said normal skin tissue associated with normal epidermal tissue is further characterized by the following features:

1 spectral pattern of sequentially expressed mean peak values and/or intensities of nucleic acids as *(965 cm^{-1}) < (1071,1084/1085,1095 cm^{-1})* versus to any between [0.5:1] and [0.7:1];

the numerical ranges of mean values of DNA peak at 965 cm^{-1} within 0.15-0.24;

the numerical ranges of mean values of the maximum peak in DNA/RNA triad within 0.22-0.47;

the numerical ranges of calculated intensities of DNA peak at 965 cm^{-1} within 0.38-0.51;

the numerical ranges of calculated intensities of the maximum peak in DNA/RNA triad within 0.49-1.00;

1 spectral pattern of sequentially expressed mean peak values and/or intensities of proteins as *(1245cm^{-1}) < (1310 cm^{-1}) > (1390 cm^{-1}) < (1450 cm^{-1}) < (1540 cm^{-1}) < (1650 cm^{-1})* versus to any between [0.5:1] and [0.7:1];

the numerical ranges of mean values of protein peak at 1310 cm-1 within 0.5;

the numerical ranges of mean values of protein peak at 1390 cm-1 within 0.3-0.4;

the numerical ranges of mean values of protein peak at 1450 cm-1 within 0.5;

the numerical range of mean values of protein peak at 1540 cm-1 within 0.8; and/or

characteristic proportional ratio within [0.5:1] — [0.7:1] to be not dependent on the grade of activity level of expressed nucleic acids and proteins in sequential patterns of nucleic acids and proteins.

10. The method of any of Claim 1-3, further comprising one of the following:

comparing any received spectral patterns of sequentially expressed nucleic acids and proteins with referenced spectral sequential patterns of nucleic acids and proteins to be specific for benign, premalignant or malignant tumours of skin tissue in association with certain skin tumour type, 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 tumourous skin tissues, characteristic for tumour progression towards benign neoplastic skin disease associated with benign compound nevus; 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 tumourous skin tissue, characteristic for tumour progression towards premalignant neoplastic skin disease associated with Bowen's disease; 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 tumourous skin tissue, characteristic for tumour progression towards malignant neoplastic skin disease associated with malignant melanoma, or associated with basal cell carcinoma, or associated with squamous cell carcinoma; 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 malignant melanoma to be characteristic for cancer progression in the same patient, wherein previously received spectral sequential patterns of nucleic acids and proteins were characteristic for benign tumourous skin tissue associated with benign compound nevus, in comparison to normal skin; or

further analysing received spectral sequential patterns of 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 squamous cell carcinoma to be characteristic for cancer progression in the same patient, wherein previously received spectral sequential patterns of nucleic acids and proteins were characteristic for premalignant tumourous skin tissue associated with Bowen's disease, in comparison to normal skin.

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

12. The method of Claims 1-10, wherein DNA/RNA triad peak (1071, 1084/1085, 1095 cm^{-1}) is defined by the maximum peak in a triad.

13. The method of Claims 1-10, wherein the numerical values of peak values of expressed proteins in FTIR spectra are numerically equalled to numercial values of calculated intensities of corresponding protein peaks in infrared spectra normalized to amide I at 1650 cm^{-1}.

14. The method of Claims 1-10, which is also applicable to analyse and characterize human skin carcinogenesis on other benign, premalignant and malignant skin tumours, than those selected from the group of benign compound nevus, Bowen's disease, malignant melanoma, squamous cell carcinoma and basal cell carcinoma.

15. The method of Claims 1-10, which is also used in a tissue sample from other organ tissue, than skin tissue.

ABSTRACT

The invention presents methods of using Fourier transform infrared (FTIR) microspectroscopy for common and specific characterization of carcinogenesis in human skin tumours. The invention provides the user with the method to analyse in patterns intra- and inter-molecular interactions for nucleic acids and proteins sequentially expressed in infrared (IR) spectra of human benign, premalignant and malignant skin tumours towards understanding the molecular, cellular and tissue changes that occur during skin carcinogenesis. More particularly, presented analytical method has the advantage to simultaneously observe DNA-RNA, DNA-DNA, DNA-protein and protein-protein interactions in patterns by means of their sequentially expressed interacting activity levels within one type of tumour and between different types of tumours, both commonly and specifically, with further indication of the grade of activity in benign, premalignant and malignant skin tissue cells.