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 cancerous 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 cancerous 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, 1084/1085, 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, 1390 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 expressed in infrared (IR) spectra of epidermal skin 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 cancerous tissue samples. 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 cancers, 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 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 basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma (MM), that have been used as examples for the user of invention. To further provide the user with the invention steps, Bowen's disease, has been chosen as an example of precancerous skin lesion, well-known as intraepidermal 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 melnoma (MM) (3 patients); from 4 patients with benign tumours: benign compound nevus (4 patients); from 3 patients with precancers: Bowen's disease (3 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, Bowen's disease and benign compound nevus, in comparison to IR spectra from healthy subjects:

- calculation of mean values of each determined nucleic acids peaks
- calculation of mean values of each determined protein (specific and non-specific) peak
- a comparison of the intensities of all determined nucleic acids peaks in the 900-1300 cm^{-1} region with the intensity of the peak at about 1245 cm^{-1} by calculating the following intensity peaks ratios: $I_{(965)}/I_{(1245)}$, $I_{(1055)}/I_{(1245)}$, $I_{(\text{max peak among DNA/RNA triad peaks})}/I_{(1245)}$.
- a comparison of 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 about 1650 cm^{-1} by calculating the following peaks ratios: $I_{(1310)}/I_{(1650)}$, $I_{(1390)}/I_{(1650)}$, $I_{(1450)}/I_{(1650)}$, $I_{(1540)}/I_{(1650)}$.
- a calculation of the level of activity of DNA-RNA vs. DNA-protein interactions by the following ratio: $I_{(\text{sum mean DNA/RNA triad peaks})}/I_{(1245)}$.
- a comparison of the level of activity of multiplet (DNA/RNA) with the level(s) of activity of the peaks in DNA/RNA triad.

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

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).

2 BCC patterns in 6 patients, based on expressed mean values vs. proportional ratios:

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

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]

1 BCC pattern 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)* (independent on the grade of activity)

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 patterns in 4 patients, based on expressed mean values vs. proportional ratios:
965mean < 1055mean > DNA/RNA triad max peak mean vs. [1:1]
965mean < 1055mean < DNA/RNA triad max peak mean vs. [0.9:1]
965mean > 1055mean < DNA/RNA triad max peak mean 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]

2 SCC patterns 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)* (for high activity levels of the peak at about 1055 cm-1)
- *I (965)/I(1245) > I(1055)/I(1245) < I (DNA/RNA triad max peak)/I (1245)* (for low activity levels of the peak at about 1055 cm-1)

1) 0.91 < 1.00 > 0.99 vs. [1:1]

2) 0.70 < 0.81 > 0.78 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 patterns in 3 patients, based on expressed mean values vs. proportional ratios:

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

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 patterns 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 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 BD pattern in 3 patients, based on mean values:

- *965mean < 1055mean < DNA/RNA triad max peak mean* (independent on the grade of activity)

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 pattern in 3 patients, based on the intensity ratios: *I (965)/I(1245) < I (1055)/I(1245) < I (DNA/RNA triad max peak)/I(1245)* (independent on the grade of activity)

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 pattern in 4 patients, based on expressed mean values vs. proportional ratios:

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

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 pattern 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)* (independent on the grade of activity)

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)

HS pattern, based on expressed mean values vs. proportional ratios:

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

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 pattern, 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, 1084, 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 $I_{965} < I_{1055} < I_{\text{max level DNA/RNA triad peak}}$ 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 $I_{965} < I_{1055} > I_{\text{max level DNA/RNA triad peak}}$ 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 $I_{965} > I_{1055} < I_{\text{max level DNA/RNA triad peak}}$ 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)* $< I_{\text{max level DNA/RNA triad peak}}$ 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

EXAMPLE 1

BCC

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

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

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. BOWEN's DISEASE (BD)

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. BENIGN COMPOUND NEVUS (BCN)

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 > **0.2 < 0.3 = 0.3** < 0.7 < 1.0 vs. [0.7:1]
5) 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: **0.4 = 0.4 = 0.4** < 0.7 < 1.0	vs.	[1:1]
2) BCC: **0.4 = 0.4 = 0.4** < 0.7 < 1.0	vs.	[1:1]
3) BCC: **0.4 < 0.5 = 0.5** < 0.8 < 1.0	vs.	[1:1]
4) SCC: **0.6 < 0.7 = 0.7** < 0.9 < 1.0	vs.	[1:1]
5) SCC: **0.4 = 0.4 = 0.4** < 0.7 < 1.0	vs.	[0.9:1]
6) MM: **0.4 < 0.5 = 0.5** < 0.8 < 1.0	vs.	[0.8:1]
7) MM: **0.3 < 0.4 = 0.4** < 0.8 < 1.0	vs.	[0.8:1]
8) BCC: **0.2 < 0.3 = 0.3** < 0.7 < 1.0	vs.	[0.8:1]
9) BCC: **0.2 < 0.3 = 0.3** < 0.7 < 1.0	vs.	[0.8:1]
10) BCC: **0.2 < 0.3 = 0.3** <0.7 < 1.0	vs.	[0.8:1]
11) SCC: **0.2 < 0.3 = 0.3** < 0.6 < 1.0	vs.	[0.7:1]
12) SCC: **0.2 = 0.2 = 0.2** < 0.6 < 1.0	vs.	[0.7:1]
13) MM: **0.4 < 0.5 < 0.5** < 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 cancers, generally indicating low, medium and high activity levels in cells in tissues.

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CLAIMS

1. A method of using Fourier transform infrared microspectroscopy for common and specific characterization of carcinogenesis in human skin tumors, comprising of:
(i) comparative assessment of expressed mean values for nucleic acids and proteins, specific and non-specific, in FTIR spectra from epidermis of benign, premalignant and malignant skin lesions
(ii) comparative calculation of the intensity ratios for all assigned peaks for nucleic acids vs. the peak at about 1245 cm-1 in FTIR spectra of benign, premalignant and malignant skin lesions
(iii) comparative calculation of the intensity ratios for all assigned peaks for proteins, specific and non-specific, vs. the amide I peak at about 1650 cm-1 in FTIR spectra from epidermis of benign, premalignant and malignant skin

lesions

(iv) determination of a pattern, both common and/or specific, based on comparative assessment of expressed mean values, for nucleic acids in FTIR spectra from epidermis of benign, premalignant and malignant skin lesions, in comparison to a determined pattern of healthy epidermis

(v) determination of a pattern, common for benign, premalignant and malignant skin tumours, based on comparative calculation of the intensity ratios, for nucleic acids and proteins in FTIR spectra of benign, premalignant and malignant lesions, in comparison to determined pattern of healthy epidermis

(vi) determination of a pattern, specific for each type of pathology, based on comparative calculation of the intensity ratios, for nucleic acids and proteins in FTIR spectra from epidermis of benign, premalignant and malignant skin lesions, in comparison to determined pattern of healthy epidermis.

2. A comparative analytical method, comprised of steps in Claim 1, for an individual patient and between the patients with any type of benign, premalignant and malignant skin lesions

3. A comparative analytical method, comprised of steps in Claim 1, for one type of pathology, related to any analyzed benign, premalignant and malignant skin lesions

4. A comparative analytical method, comprised of steps in Claim 1, for different types of epidermal skin cancers

5. A method according to Claims 1-4, for indication of the grade of activity in neoplastic cells of measured skin tumors, comprising the following steps:

- calculation of the level of activity of DNA-RNA vs. DNA-protein interactions by the following proportional ratio: $I_{(\text{sum mean DNA/RNA triad peaks})}/I_{(1245)}$
- calculation of mean values of each assigned nucleic acids peak, in comparison to a proportional ratio, i.e. sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}
- calculation of mean values of each assigned protein (specific and nonspecific) peak, in comparison to a proportional ratio, i.e. sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}
- a comparison of the intensities of all determined nucleic acids peaks in the 900-1300 cm^{-1} region with the intensity of the peak at about 1245 cm^{-1} by calculating the following intensity peaks ratios: $I_{(965)}/I$

$I_{(1245)}$, $I_{(1055)}/I_{(1245)}$, $I_{(\text{max peak among DNA/RNA triad peaks})}/I_{(1245)}$, in comparison to a proportional ratio, i.e. sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}

- a comparison of the intensities of all determined peaks for specific and non-specific proteins in the 1300-1700 cm^{-1} region with the intensity of amide I peak at about 1650 cm^{-1} by calculating the following peaks ratios: $I_{(1310)}/I_{(1650)}$, $I_{(1390)}/I_{(1650)}$, $I_{(1450)}/I_{(1650)}$, $I_{(1540)}/I_{(1650)}$, in comparison to a proportional ratio, i.e. sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}
- a comparison of the level of activity of multiplet (DNA/RNA at about 1055 cm-1) with the level(s) of activity of the peaks in DNA/RNA triad (at about 1071, 1084, 1095 cm-1)

6. A comparative method of analysing a systematic progression of the grade of activity between benign, premalignant and malignant skin tissue cells, comprising of steps in Claim 5

7. A method of analysing a systematic progression of the grade of activity in malignant skin tissue cells in different types of pathologies, comprising of steps in Claim 5

8. A method of analysing a systematic progression of the grade of activity in malignant skin tissue cells within one type of pathology, comprising of steps in Claim 5

CLAIMS

1. A method of using Fourier transform infrared microspectroscopy for common and specific characterization of carcinogenesis in human skin tumors, comprising of:
(i) comparative assessment of expressed mean values for nucleic acids and proteins, specific and non-specific, in FTIR spectra from epidermis of benign, premalignant and malignant skin lesions
(ii) comparative calculation of the intensity ratios for all assigned peaks for nucleic acids vs. the peak at about 1245 cm-1 in FTIR spectra of benign, premalignant and malignant skin lesions
(iii) comparative calculation of the intensity ratios for all assigned peaks for proteins, specific and non-specific, vs. the amide I peak at about 1650 cm-1 in FTIR spectra from epidermis of benign, premalignant and malignant skin lesions
(iv) determination of a pattern, both common and/or specific, based on comparative assessment of expressed mean values, for nucleic acids in FTIR spectra from epidermis of benign, premalignant and malignant skin lesions, in comparison to a determined pattern of healthy epidermis
(v) determination of a pattern, common for benign, premalignant and malignant skin tumours, based on comparative calculation of the intensity ratios, for nucleic acids and proteins in FTIR spectra of benign, premalignant and malignant lesions, in comparison to determined pattern of healthy epidermis
(vi) determination of a pattern, specific for each type of pathology, based on comparative calculation of the intensity ratios, for nucleic acids and proteins in FTIR spectra from epidermis of benign, premalignant and malignant skin lesions, in comparison to determined pattern of healthy epidermis
2. A comparative analytical method, comprised of steps in Claim 1, for an individual patient and between the patients with any type of benign, premalignant and malignant skin lesions
3. A comparative analytical method, comprised of steps in Claim 1, for one type of pathology, related to any analyzed benign, premalignant and malignant skin lesions
4. A comparative analytical method, comprised of steps in Claim 1, for different types of epidermal skin cancers
5. A method according to Claims 1-4. for indication of the grade of activity

in neoplastic cells of measured skin tumors, comprising the following steps:

- calculation of the level of activity of DNA-RNA vs. DNA-protein interactions by the following proportional ratio: $I_{(\text{sum mean DNA/RNA triad peaks})}/I_{(1245)}$.
- calculation of mean values of each assigned nucleic acids peak, in comparison to a proportional ratio, i.e. sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}
- calculation of mean values of each assigned protein (specific and nonspecific) peak, in comparison to a proportional ratio, i.e. sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}
- a comparison of the intensities of all determined nucleic acids peaks in the 900-1300 cm^{-1} region with the intensity of the peak at about 1245 cm^{-1} by calculating the following intensity peaks ratios: $I_{(965)}/I_{(1245)}$, $I_{(1055)}/I_{(1245)}$, $I_{(\text{max peak among DNA/RNA triad peaks})}/I_{(1245)}$, in comparison to a proportional ratio, i.e. sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}
- a comparison of the intensities of all determined peaks for specific and non-specific proteins in the 1300-1700 cm^{-1} region with the intensity of amide I peak at about 1650 cm^{-1} by calculating the following peaks ratios: $I_{(1310)}/I_{(1650)}$, $I_{(1390)}/I_{(1650)}$, $I_{(1450)}/I_{(1650)}$, $I_{(1540)}/I_{(1650)}$, in comparison to a proportional ratio, i.e. sum mean DNA/RNA triad peaks vs. 1245 cm^{-1}
- a comparison of the level of activity of multiplet (DNA/RNA at about 1055 cm-1) with the level(s) of activity of the peaks in DNA/RNA triad (at about 1071, 1084, 1095 cm-1)

6. A comparative method of analysing a systematic progression of the grade of activity between benign, premalignant and malignant skin tissue cells, comprising of steps in Claim 5

7. A method of analysing a systematic progression of the grade of activity in malignant skin tissue cells in different types of pathologies, comprising of steps in Claim 5

8. A method of analysing a systematic progression of the grade of activity in malignant skin tissue cells within one type of pathology, comprising of steps

in Claim 5

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 **a method** for non-destructive characterization of **the multistage nature of** carcinogenesis in **benign, premalignant and malignant human** skin **tumourous** tissues **with the use of** Fourier transform infrared (FTIR) microspectroscopy. More specifically, the invention provides with an analytical method for observing **evolutionary expression of nucleic acids and proteins, alone and in interactions, determined in IR spectra through three developmental stages during human skin carcinogenesis. Method analyses intra- and inter-molecular interactions for nucleic acids and proteins expressed in DNA and protein sequences observed in FTIR spectrum of a tissue sample, commonly and specifically expressed in IR spectra of each type of pathology,** that **further** finds use for indication of the grade of neoplastic activity in cells in human skin cancerous tissues. **More, method provides with molecular models of tissue specificity – benign, premalignant and malignant – to represent set of important molecular features of the interacting molecules of DNA and proteins for tissue specification, qualitatively and quantitatively. Tissue- -specific expression profiles with described patterns accurately predict patterns for benign, premalignant and malignant tissues against healthy skin, in order to construct a model of developmental stages of human skin carcinogenesis by FTIR microspectroscopy.**

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.

Carcinogenesis or oncogenesis or tumorigenesis is the actual formation of a cancer, whereby normal cells are transformed into cancer cells. The process is characterized by a progression of changes at the cellular, genetic, and epigenetic level that ultimately reprogram a cell to undergo uncontrolled cell division, thereby forming a malignant mass. The process is characterized by a progression of changes at the cellular, genetic, and epigenetic level that ultimately reprogram a cell to undergo uncontrolled cell

division, thereby forming a malignant mass. Cell division is a physiological process that occurs in almost all tissues and under many circumstances. Under normal circumstances, the balance between proliferation and programmed cell death, usually in the form of apoptosis, is maintained by regulation of both processes to ensure the integrity of tissues and organs. Mutations and epimutations in DNA lead to cancer. More than one mutation is necessary for carcinogenesis. In fact, a series of several mutations to certain classes of genes is usually required before a normal cell will transform into a cancer cell.

Cancer is fundamentally a disease of regulation of tissue growth. In order for a normal cell to transform into a cancer cell, genes that regulate cell growth and differentiation must be altered. Genetic and epigenetic changes can occur at many levels, from gain or loss of entire chromosomes, to a mutation affecting a single DNA nucleotide, or to silencing or activating a microRNA that controls expression of 100 to 500 genes. There is a diverse classification scheme for the various genomic changes that may contribute to the generation of cancer cells. Many of these changes mutations, or changes in the nucleotide sequence of genomic DNA.

Skin tumour, as any other tumour, goes through three developmental stages. First, a benign tumour occurs, then it grows into interstitial, "local" cancer, which later spreads and forms infiltrating malignant neoplasms.

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

For many years the mouse skin model provided a possibility to study multistage carcinogenesis mechanisms [1], but without direct relevance of this model system to human skin cancers. In the mouse skin model the first stage is thought to involve the interaction of a tumour initiator with the genetic material of stem cells leading to an irreversible alteration in some aspect of growth control and/or differentiation. A number of growth factors have recently been found to be increased during tumour promotion and may be responsible for the increase in cell proliferation. An inhibition of cell-cell communication and stimulation of differentiation of non-initiated cells appear to be important indirect mechanisms of further expanding the initiated cell population. The appearance of alpha-glutamyl transpeptidase and keratin 13, and the lack of expression of keratins 1 and 10 were found to be good markers for skin tumor progression. [2]

However, none of the existing methods do permit studying mechanisms of cancerogenesis directly on **skin tissue of human beings** and do not answer to all existing questions at present. **When analyzing bioptic material, clinicians sometimes find difficulty in determining the**

tumour development stage, particuarly in a transition state, by existing standards in the hospital.

FT-IR microspectroscopy holds great promise not only as a method of ascertaining changes in the chemistry of the neoplastic cells but also as a diagnostic tool, especially for early stages of carcinogenesis not detectable by other means [4-7].

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, **including molecules of DNA, proteins, carbohydrates, water, etc. [4]**

Classic spectroscopic analysis by FTIR microspectroscopy demonstrated marked differences in the mid-FTIR spectra between normal, precancerous and cancerous tissues 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 **[5].**

Studies by FTIR microspectroscopy on 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, 1084/1085, 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, 1390 and 1450 cm-1; the amide II vibration at about 1540 cm-1; and the amide I vibration at about 1650 cm-1 **[5-6]. FTIR spectra from epidermal skin cancers showed that the molecules of DNA and proteins can be studied in intra- and inter-molecular interactions by FTIR microspectroscopy with high sensitivity and find use for diagnosis of skin cancers [6].** Nevertheless, the problem has not been yet addressed towards common and specific characterization of carcinogenesis in human skin tumours by FTIR microspectroscopy **for observing evolutionary changes at the molecular levels from early stages of skin carcinogenesis. The mechanism by which a cell progress from normalcy to malignancy has not been understood in these studies by FTIR microspectroscopy.** For better understanding of molecular and cellular mechanisms involved in skin cancer development, there is a need for a method to directly characterize on human tissues skin 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. **Being non-destructive, FTIR micropectroscopy provides the opportunity to probe cellular changes in intact tissues.**

Therefore, it is another purpose of this invention to provide with the first **analytical method** for **common and specific characterization of human skin carcinogenesis in intact skin, based on objective evolutionary and comparative assessment of molecular changes of** nucleic acids and proteins in intra- and inter-molecular interactions expressed **in FTIR** spectra of benign, premalignant and malignant human skin tumourous tissues, **especially for early stages of carcinogenesis not detectable by other means.**

SUMMARY OF THE INVENTION

The invention presents a method of common and specific characterization of developmental stages of human skin carcinogenesis in intact benign, premalignant and malignant human skin tumourous tissues based on DNA and protein patterned recognized sequence analysis in the mid-IR spectra by FTIR microspectroscopy, the method comprising of:

(i) evolutionary observation of expression of nucleic acids and proteins in DNA and protein sequences, thus for identification of patterns of DNA and proteins sequences in benign, premalignant and malignant human skin tumourous tissues; and

(ii) tissue-specific recognition of patterns of DNA and protein sequences, thus distinctive for benign, premalignant and malignant human skin tumourous tissues; and

(iii) tumour-specific recognition of patterns of DNA and protein sequences, thus *distinctive for benign, premalignant and malignant skin lesions*

wherein said DNA and protein patterned sequence recognitions are in comparison with patterns, specific for human healthy epidermal tissue.

The solution to said analytical problem is achieved by providing the embodiments characterized in the claims.

In a first aspect, the invention provides a method of using FTIR microspectroscopy for characterization of common and specific carcinogenesis in human biopsied skin **tumourous** tissue samples. The sample can be comprised of normal, benign, precancerous or cancerous cells on a **human** 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 IR spectra of benign, premalignant and malignant skin **tumourous tissues**, 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 cancer and between different types of cancers, to assess the activity of neoplastic cells in each patient and between the patients by FTIR microspectroscopy.

In a second aspect, the invention provides with the method of recognizing tissue- and tumour-specific patterns of DNA and protein sequences in FTIR spectra.

By DNA sequence is meant a sequence that comprises a range of frequency bands at ~ 965, 1055, 1071, 1084/1085, 1095 cm-1, specific for nucleic acids in the FTIR spectra.

By protein sequence is meant a sequenc that comprises a range frequency bands at ~ 1310, 1390, 1450, 1084/1085, 1095 cm-1, specific for proteins in the FTIR spectra.

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 basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma (MM), that have been used as examples for the user of invention. To provide the user with the invention steps **for earlier stages of carcinogenesis**, Bowen's disease, has been chosen as an example of precancerous skin lesion, well-known as intraepidermal carcinoma *in situ.* **For the earliest stage of carcinogenesis,** benign compound nevus has been chosen as a common example of benign skin tumour in the presented invention. **A nevus, or mole, is a benign growth of the pigment forming cells. 50 % of MMs arise from moles.**

The invention further indicates how to describe common and specific features in examined tissue towards skin cancer development by means of comparing expressed mean values and mean intensities of each frequency band, detected in DNA and protein sequences, with proportional ratios, to indicate the grade of activity in cells of benign, premalignant and malignant cancerous tissues, as well to analyse a systemic progression of the grade of activity within one type of tumour and between different types of tumour.

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 **intact** 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.

The invented method is an analytical method of common and specific characterization of developmental stages of human skin carcinogenesis in intact benign, premalignant and malignant human skin tumourous tissues based on DNA and protein pattern-recognized sequence analysis in the mid-IR spectra by FTIR microspectroscopy, the method comprising of:

(i) evolutionary observation of expression of nucleic acids and proteins in DNA and protein sequences, thus for identification of patterns of DNA and protein sequences in benign, premalignant and malignant human skin tumourous tissues; and

(ii) tissue-specific recognition of patterns of DNA and protein sequences, thus distinctive for benign, premalignant and malignant human skin tumourous tissues; and

(iii) tumour-specific recognition of patterns of DNA and protein sequences, thus distinctive for benign, premalignant and malignant skin lesions

wherein said DNA and protein patterned sequence recognitions are always compared with patterns, specific for intact human healthy epidermal tissue.

The invented method recognises tissue- and tumour-specific patterns of DNA sequences in FTIR spectra, that are characterized by:

(i) DNA sequence detection by means of determination of DNA-specific frequency bands over a range of known bands at about 965 cm^{-1}, 1055 cm^{-1}, and **at about 1071 cm^{-1}, 1084/1085 cm^{-1}, 1095 cm^{-1} in the DNA/RNA triad**, to find the following **DNA sequence** patterns, as:

(a) **a sequence as of** "DNA(965), DNA/RNA triad (1071, 1084/1085, 1095)", distinctive for healthy tissue;

(b) **a sequence as of** "DNA(965), DNA/RNA(1055), DNA/RNA triad (1071, 1084/1085, 1095)", distinctive for benign, premalignant and malignant cancerous tissue;

(ii) mean value calculation of each frequency band, detected in DNA sequence **as of** "DNA(965), DNA/RNA(1055), DNA/RNA triad (1071, 1084/1085, 1095)", to find further patterns, as:

(a) "DNA(965_{mean}) < DNA/RNA(1055_{mean}) < DNA/RNA triad (1071, 1084/1085/$1095_{max\ peak\ mean}$)", characteristic for skin benign tumours;

(b) "DNA(965_{mean}) < DNA/RNA(1055_{mean}) < DNA/RNAtriad (1071, 1084/1085/$1095_{max\ peak\ mean}$)", characteristic for skin premalignant tumours;

(c) "DNA(965_{mean}) < DNA/RNA(1055_{mean}) < DNA/RNAtriad (1071, 1084/1085/$1095_{max\ peak\ mean}$)", and

"DNA(965_{mean}) < DNA/RNA(1055_{mean}) > DNA/RNAtriad (1071, 1084/1085/$1095_{max\ peak\ mean}$)", and

"DNA(965_{mean}) > DNA/RNA(1055_{mean}) < DNA/RNAtriad (1071, 1084/1085/$1095_{max\ peak\ mean}$)",

characteristic for skin malignant tumours.

(iii) mean intensity calculation of each frequency band detected in DNA sequence to find further patterns, as:
(a) "DNA($I_{(965mean)}/I_{(1245mean)}$) < DNA/RNA($I_{(1055mean)}/I_{(1245mean)}$) < DNA/RNAtriad ($I_{(DNA/RNA\ triad\ max\ peak\ mean)}/I_{(1245mean)}$)", characteristic for skin benign tumours;

(b) "DNA($I_{(965mean)}/I_{(1245mean)}$) < DNA/RNA($I_{(1055mean)}/I_{(1245mean)}$) < DNA/RNAtriad ($I_{(DNA/RNA\ triad\ max\ peak\ mean)}/I_{(1245mean)}$)", characteristic for skin premalignant tumours;

(c) "DNA($I_{(965mean)}/I_{(1245mean)}$) < DNA/RNA($I_{(1055mean)}/I_{(1245mean)}$) < DNA/RNAtriad ($I_{(DNA/RNA\ triad\ max\ peak\ mean)}/I_{(1245mean)}$)", as well

"DNA($I_{(965mean)}/I_{(1245mean)}$) < DNA/RNA($I_{(1055mean)}/I_{(1245mean)}$) > ($I_{(DNA/RNA\ triad\ max\ peak\ mean)}/I_{(1245mean)}$)", as well

"DNA($I_{(965mean)}/I_{(1245mean)}$) > DNA/RNA($I_{(1055mean)}/I_{(1245mean)}$) < ($I_{(DNA/RNA\ triad\ max\ peak\ mean)}/I_{(1245mean)}$)",

characteristic for skin malignant tumours.

(iv) calculation of proportional ratio to characterize epidermal tissue towards skin cancer development within the following ranges, as:

(a) [0.5:1] - [0.7:1], specific for healthy epidermis;

(b) [0.7:1] - [0.9:1], specific for skin benign tumours;

(c) [0.8:1] - [0.9:1], specific for skin premalignant tumours;

(d) [0.7:1]-[1:1], specific for skin malignant tumours, BUT dependent on the type of tumour.

The invented method recognises tissue- and tumour-specific patterns of protein sequences in FTIR spectra, that are characterized by:

(i) mean value calculation of each protein-specific frequency band in detected protein sequence, for descriptive and non-descriptive proteins at about 1310 cm^{-1}, 1390 cm^{-1}, 1450 cm^{-1}, 1540 cm^{-1} and 1650 cm^{-1}, wherein

(a) expressed mean values of non-descriptive, i.e. non-specific, protein at about 1310 cm^{-1} determine the starting level, i.e. activity level, in detected protein sequences from healthy, benign, premalignant and malignant cancerous tissues

(b) mean values of non-descriptive, i.e. non-specific, protein at about 1310 cm^{-1} in protein sequences are ranged in strong correlation with gradation in proportional ratios

(c) mean values of non-descriptive, i.e. non-specific protein at about 1310 cm^{-1} in protein sequences are always at similar values expressed by activity levels of DNA/amide III peak at about 1245 cm^{-1}

(d) mean values of non-desciptive, i.e. non-specific, proteins at about 1390 cm^{-1} and 1450 cm^{-1} are expressed equally in protein sequences of premalignant and malignant cancerous tissues, but not of benign cancerous tissue vs. [0.9:1]

(e) mean values of specific proteins at about 1540 cm^{-1} are expressed in strong correlation with gradation in proportional ratios, but differ for benign, premalignant and malignant cancerous tissues

(ii) a comparative analysis of the activity of expressed mean values in protein sequences related to benign, premalignant and malignant cancerous tissues, as:

(a) specific for benign skin tumours, with reference to benign compound nevus, characterized by:

- 2 patterns of non-specific protein sequences, as "(1310) < (1390) = (1450)" and "(1310) < (1390) < (1450)"
- the pattern, "(1310) < (1390) = (1450)", is for ranged mean values that is correlated with [0.7:1] and [0.8:1] proportional ratios
- the pattern, "(1310) < (1390) < (1450)" is for ranged mean values that is in strong correlation with [0.9:1] proportional ratio
- mean values for non-specific proteins, ranged within 0.2-0.4, and mean values for specific protein at about 1540 cm^{-1}, ranged within 0.7-0.8, do not show correlation with any of the gradation in proportional ratios between [0.7:1]-[0.9:1]

(b) specific for premalignant cancerous tissue, with reference to Bowen's disease, characterized by:

- one-patterned non-specific protein sequences, as "(1310) < (1390) = (1450)"
- this pattern is strongly correlated with gradation in proportional ratios
- mean values for non-specific proteins, ranged within 0.1-0.4, and mean values for specific protein at about 1540 cm^{-1}, ranged within 0.6-0.8, increase with gradation increment in proportional ratios from [0.7:1] to [1:1]

(c) specific for malignant cancerous tissue,

with reference to basal cell carcinoma, characterized by:

- 2 patterns of non-specific protein sequences, as "(1310) = (1390) = (1450)" and "(1310) < (1390) = (1450)"

- the pattern "(1310) = (1390) = (1450)" is only correlated with the gradation in [1:1] proportional ratios
- mean values for non-specific proteins, ranged within 0.2-0.5 in the pattern as of "(1310) < (1390) = (1450)", and mean values for specific proteins at about 1540 cm^{-1}, ranged within 0.7-0.8, are expressed in strong correlation with gradation increment in proportion ratios from [0.8:1] to [1:1]

with reference to squamous cell carcinoma, characterized by:

- 2 patterns of non-specific protein sequences, as "(1310) = (1390) = (1450)" and "(1310) < (1390) = (1450)"
- these patterns are not correlated with the gradation in proportional ratios
- high-ranged mean values for non-specific proteins within 0.2-0.7, and mean values for specific proteins at about 1540 cm^{-1} ranged within 0.6-0.9, are expressed in strong correlation with gradation increment in proportional ratios from [0.7:1] to [1:1]

with reference to malignant melanoma, characterized by:

- one-patterned non-specific protein sequences, as "(1310) < (1390) = (1450)"
- low-ranged mean values for non-specific proteins within 0.3-0.5 are expressed with mean values of 0.8 for specific proteins at about 1540 cm^{-1}, that are higher than for benign tumour

The use of the invented method is to further indicate common and specific features in examined tissue towards skin cancer development by means of comparing expressed mean values and mean intensities of each frequency band, detected in DNA and protein sequences, with proportional ratios, to indicate the grade of activity in cells of benign, premalignant and malignant cancerous tissues, as well to analyse a systemic progression of the grade of activity within one type of tumour and between different types of tumour, comprising of:

(i) detection of the lowest and the highest mean values and intensities in all patterned DNA sequences specific to each kind of cancerous tissue to commonly characterize cancer progression, as:

(a) in benign cancerous tissue with reference to benign compound nevus

with the *lowest mean values*,

as "DNA(0.12) < DNA/RNA(0.17) < DNA/RNA triad(0.19) vs. [0.7:1]

and the *lowest intensities*,

as "DNA(0.48) <DNA/RNA(0.68) < DNA/RNA triad(0.76) vs. [0.7:1];

with the *highest mean values*,

as "DNA(0.18) < DNA/RNA(0.24) < DNA/RNA triad(0.27) vs. [0.9:1],

and with the *highest intensities*,

as "DNA(0.56) <DNA/RNA(0.75) < DNA/RNA triad(0.84) vs. [0.9:1].

(b) in precancerous tissue with reference to Bowen's disease

with the *lowest mean values*,

as "DNA(0.09) < DNA/RNA(0.15) < DNA/RNA triad(0.18) vs. [0.8:1],

and the *lowest intensities*,

as "DNA(0.41) <DNA/RNA(0.68) < DNA/RNA triad(0.82) vs. [0.8:1];

with the *highest mean values*,

as "DNA(0.23) < DNA/RNA(0.26) < DNA/RNA triad(0.29) vs. [0.9:1],

and with the *highest intensities*,

as "DNA(0.70) <DNA/RNA(0.79) < DNA/RNA triad(0.88) vs. [0.9:1].

(c) in malignant cancerous tissue,

with reference to squamous cell carcinoma,

with the *lowest mean values*,

as "DNA(0.19) > DNA/RNA(0.13) < DNA/RNA triad(0.16) vs. [0.7:1],

and with the *lowest intensities*,

as "DNA(0.41) < DNA/RNA(0.77) > DNA/RNA triad(0.71) vs. [0.7:1].

with the *highest mean values*,

as "DNA(0.60) < DNA/RNA(0.66) > DNA/RNA triad(0.65) vs. [1:1],

and with *the highest intensities*,

as "DNA(0.91) < DNA/RNA(1.00) > DNA/RNA triad(0.99) vs. [1:1];

with reference to basal cell carcinoma,

with the *lowest mean values*,

as "DNA(0.08) < DNA/RNA(0.14) < DNA/RNA triad(0.16) vs. [0.8:1],

and with the *lowest intensities*,

as "DNA(0.38) < DNA/RNA(0.67) < DNA/RNA triad(0.76) vs. [0.8:1];

with the *highest mean values*,

as "DNA(0.35) < DNA/RNA(0.44) < DNA/RNA triad(0.45) vs. [1:1],

and with the *highest intensities*,

as "DNA(0.75) < DNA/RNA(0.94) < DNA/RNA triad(0.96) vs. [1:1].

with reference to malignant melanoma,

with the *lowest/highest mean values*,

as "DNA(0.23) < DNA/RNA(0.26) < DNA/RNA triad(0.30) vs. [0.8:1],

as "DNA(0.19) > DNA/RNA(0.14) < DNA/RNA triad(0.27) vs. [0.8:1];

with the *lowest/highest intensities*,

as "DNA(0.61) < DNA/RNA(0.69) < DNA/RNA triad(0.79) vs. [0.8:1],

as "DNA(0.63) > DNA/RNA(0.47) < DNA/RNA triad(0.90) vs. [0.8:1].

(ii) correlating the lowest/highest expressed mean values and calculated intensities in detected DNA sequences with proportional ratios, to specifically characterize cancer progression within one type of tumour, as:

(a) in benign skin tumours, with reference to benign compound nevus, characterized by:

-one pattern of DNA sequence as "DNA(965) < DNA/RNA(1055) < DNA/RNA triad(1071, 1084/1085, 1095)"

-this pattern is independent on the grade of activity by proportional ratios

-this pattern is characterized in low-ranged mean values and calculated intensities when compared with premalignant and malignant skin tumours, that are well-correlated with gradation in proportional ratios

(b) in premalignant skin tumours, with reference to Bowen's disease, characterized by:

-one pattern of DNA sequence as "DNA(965) < DNA/RNA(1055) < DNA/RNA triad(1071, 1084/1085, 1095)"

-this pattern is independent on the grade of activity by proportional ratios

-this pattern is characterized by well-correlated with gradation in proprotional ratios intermediate ranges of mean values and calculated intensities, that are higher than for benign skin tumours, but lower than for malignant skin tumours

(c) in malignant skin tumours,

with reference to basal cell carcinoma, characterized by:

-2 patterns of DNA sequences: 1) "DNA(965) < DNA/RNA(1055) < DNA/RNA triad(1071, 1084/1085, 1095)"; 2) "DNA(965) < DNA/RNA(1055) > DNA/RNA triad(1071, 1084/1085, 1095)"

-DNA sequence pattern "DNA(965) < DNA/RNA(1055) < DNA/RNA triad (1071, 1084/1085, 1095)" is dominant, independent whether based on the expressed mean values or calculated intensities

-these patterns are independent on the grade of activity by proportional ratios

-these patterns are characterized in high-ranged mean values and calculated intensities that are well-correlated with gradation in proportional ratios, when compared with benign and premalignant skin tumours

with reference to squamous cell carcinoma, characterized by:

-3 patterns of DNA sequences as "DNA(965) < DNA(1055) > DNA/RNA triad", "DNA(965) < DNA(1055) < DNA/RNA triad" and "DNA(965) > DNA(1055) < DNA/RNA triad", based on expressed mean values, that are dependent on the grade of activity by proportional ratios and the acitivity of DNA/RNA peak at about 1055 cm^{-1},

-one-patterned DNA sequence "DNA(965) < DNA(1055) > DNA/RNA triad", based on calculated intensities, that are dependent on the grade of activity by proportional ratios and the acitivity of DNA/RNA peak at about 1055 cm^{-1}

-all patterns, independent whether based on expressed mean values or calculated intensities, are characterized by high ranges, that are higher in comparison to other malignant skin tumours, and are well-correlated with gradation in proportional ratios

with reference to malignant melanoma, characterized by:

-2 patterns of DNA sequences as "DNA(965) < DNA(1055) < DNA/RNA triad" and "DNA(965) > DNA(1055) < DNA/RNA triad", independent whether based on the expressed mean values or calculated intensities

-these patterns are independent on the grade of activity by proportional ratios

-these patterns are characterized by the highest levels of expressed mean values and calculated intensities vs. lower gradation in proportional ratios, when compared with the other types of malignant skin tumours

(iii) comparative protein sequence analysis for determination of systematic progression of the grade of activity between benign, premalignant and malignant skin tissue cells, as:

(a) specific to indicate a progression from premalignant to malignant cancerous tissue against healthy epidermal tissue,

characterized in protein sequences by expressed mean values in relation to gradation in proportional ratios, as :

with reference to healthy epidermal skin, i.e. HS, Bowen's disease (*in situ* cancer), i.e. BD, vs. squamous cell carcinoma (SCC)

- in relation to [0.7:1]

BD: 0.1 < 0.2 = 0.2 < 0.6 < 1.0

SCC: 0.2 = 0.2 = 0.2 < 0.6 < 1.0

SCC: 0.2 < 0.3 = 0.3 < 0.6 < 1.0

HS: 0.4 = 0.4 = 0.4 = 0.4 < 0.8 < 1.0

HS: 0.5 < 0.6 > 0.5 =0.5 < 0.8 < 1.0

-in relation to [0.9:1]

BD: 0.3 <0.4 = 0.4 < 0.8 < 1.0

SCC: 0.4 = 0.4 = 0.4 < 0.7 < 1.0

(b) specific to indicate a progression from benign to malignant cancerous tissue in known pathologies to transform from benign to malignant skin tumours against healthy epidermal tissue,

characterized in protein sequences by expressed mean values in relation to gradation in proportional ratios, as:

with reference to healthy epidermal skin, i.e. HS, benign compound nevus, i.e. BCN vs. malignant melanoma (MM)

- in relation to [0.7:1]:

BCN: 0.3 < 0.4 = 0.4 < 0.8 < 1.0

BCN: 0.2 < 0.3 = 0.3 < 0.7 < 1.0

MM: 0.2 < 0.3 = 0.3 < 0.6 < 1.0

HS: 0.4 = 0.4 = 0.4 = 0.4 < 0.8 < 1.0

HS: 0.5 < 0.6 > 0.5 =0.5 < 0.8 < 1.0

- in relation to [0.8:1]

BCN: 0.2 < 0.3 = 0.3 < 0.7 < 1.0

MM: 0.4 <0.5 = 0.5 < 0.8 < 1.0

MM: 0.3 < 0.4 = 0.4 < 0.8 < 1.0

(c) specific to indicate a progression from benign to premalignant, from premalignant to malignant skin cancerous tissue, against healthy epidermal tissue,

characterized in protein sequences by expressed mean values in relation to gradation in proportional ratios, as:

-in relation to [0.7:1]

BCN: 0.2 < 0.3 = 0.3 < 0.7 < 1.0

BCN: 0.3 < 0.4 = 0.4 < 0.8 < 1.0

BD: 0.1 < 0.2 = 0.2 < 0.6 < 1.0

SCC: 0.2 = 0.2 = 0.2 < 0.6 < 1.0

SCC: 0.2 < 0.3 = 0.3 < 0.6 < 1.0

MM: 0.4 < 0.5 = 0.5 < 0.8 < 1.0

HS: 0.4 = 0.4 = 0.4 = 0.4 < 0.8 < 1.0

HS: 0.5 < 0.6 > 0.5 =0.5 < 0.8 < 1.0

in relation to [0.8:1]

BCN: 0.2 < 0.3 = 0.3 < 0.7 < 1.0

BD: 0.2 < 0.3 = 0.3 < 0.7 < 1.0

BCC: 0.2 < 0.3 = 0.3 < 0.7 < 1.0

MM: 0.3 < 0.4 = 0.4 < 0.8 < 1.0

MM: 0.4 < 0.5 = 0.5 < 0.8 < 1.0

in relation to [0.9:1]

BCN: 0.2 < 0.3 < 0.4 < 0.7 < 1.0

BD: 0.3 < 0.4 = 0.4 < 0.8 < 1.0

SCC: 0.4 = 0.4 = 0.4 < 0.7 < 1.0

in relation to [1:1]

BCC: 0.4 = 0.4 = 0.4 < 0.7 < 1.0

BCC: 0.4 = 0.4 = 0.4 < 0.8 < 1.0

BCC: 0.4 < 0.5 = 0.5 < 0.8 < 1.0

SCC: 0.6 < 0.7 = 0.7 < 0.9 < 1.0

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.

Raw (unprocessed) 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 **raw** 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 melnoma (MM) (3 patients); from 4 patients with benign tumours: benign compound nevus (4 patients); from 3 patients with precancers: Bowen's disease (3 patients); and from 4 healthy subjects.

Basic spectral analysis by FTIR microspectroscopy comprised of 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 **have been** 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

iv.

The following steps have been performed pre-processed FTIR spectra prior tissue-specific recognition of patterns of DNA and protein sequences in FTIR spectra for determination of the activity levels for nucleic acids and proteins:

* calculation of mean values of each determined nucleic acids peaks
* calculation of mean values of each determined protein (specific and non-specific) peak
* a comparison of the intensities of all determined nucleic acids peaks in the 900-1300 cm-1 region with the intensity of the peak at about 1245 cm-1 by calculating the following intensity peaks ratios: I(965)/I(1245), I(1055)/I(1245), I(max peak among DNA/RNA triad peaks)/I(1245).
* a comparison of 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 about 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).
* a 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).
* a comparison of the level of activity of multiplet (DNA/RNA) with the level(s) of activity of the peaks in DNA/RNA triad.

Below is a summary of the data employed from the patients with basal cell carcinoma, squamous cell carcinoma, malignant melanoma, Bowen's disease, benign compound nevus and healthy subjects, provided in examples.

INTRA- AND INTER-MOLECULAR INTERACTIONS FOR NUCLEIC ACIDS

in FTIR spectra

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for recognising DNA patterned sequences

EXAMPLES

EXAMPLE 1

HEALTHY SKIN (HS)

HS pattern, based on expressed mean values vs. proportional ratios:

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

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 pattern, 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]

EXAMPLE 2

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 pattern in 4 patients, based on expressed mean values vs. proportional ratios:

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

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 pattern 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)* (independent on the grade of activity)

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 3

BOWEN'S DISEASE (BD)

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 BD pattern in 3 patients, based on mean values:

* *965mean < 1055mean < DNA/RNA triad max peak mean* (independent on the grade of activity)

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 pattern in 3 patients, based on the intensity ratios: *I (965)/I(1245) < I(1055)/I(1245) < I (DNA/RNA triad max peak)/I(1245)* (independent on the grade of activity)

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 4

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).

2 BCC patterns in 6 patients, based on expressed mean values vs. proportional ratios:

* *965mean < 1055mean < DNA/RNA triad max peak mean*
* *965mean < 1055mean > DNA/RNA triad max peak mean*

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]

1 BCC pattern 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)* (independent on the grade of activity)

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 5

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 patterns in 4 patients, based on expressed mean values vs. proportional ratios:

965mean < 1055mean > DNA/RNA triad max peak mean vs. [1:1]
965mean < 1055mean < DNA/RNA triad max peak mean vs. [0.9:1]
965mean > 1055mean < DNA/RNA triad max peak mean 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]

2 SCC patterns 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)* (for high activity levels of the peak at about 1055 cm-1)
* *I (965)/I(1245) > I(1055)/I(1245) < I (DNA/RNA triad max peak)/I(1245)* (for low activity levels of the peak at about 1055 cm-1)

1) 0.91 < 1.00 > 0.99 vs. [1:1]
2) 0.70 < 0.81 > 0.78 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 6

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 patterns in 3 patients, based on expressed mean values vs. proportional ratios:

* *965mean > 1055mean < DNA/RNA triad max peak mean*
* *965mean < 1055mean < DNA/RNA triad max peak mean*

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 patterns 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]

RESULTS

Based on the presented Examples **2-6**, 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, 1084, 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 *I965 < I1055 < 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 *I965 < I1055 > 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 *I965 > I1055 < 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 *I965 > (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 FTIR spectra

from intact healthy, benign, premalignant and malignant human skin tissues

for recognising protein patterned sequences

EXAMPLE 1

HEALTHY SKIN (HS)

HS: 0.6 > 0.5 = 0.5 < 0.8 < 1.0 vs. [0.7 : 1]

EXAMPLE 2

BENIGN COMPOUND NEVUS (BCN) vs. MM

1) BCN: 0.3 > **0.2 < 0.3 = 0.3** < 0.7 < 1.0 vs. [0.7:1]

2) BCN: 0.3 = **0.3 < 0.4 = 0.4** < 0.8 < 1.0 vs. [0.7:1]

3) MM: 0.5 > **0.4 < 0.5 < 0.5** < 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) BCN: 0.3 > **0.2 < 0.3 = 0.3** < 0.7 < 1.0 vs. [0.8:1]

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]

EXAMPLE 3

BOWEN's DISEASE (BD) vs. SCC

1) BD: 0.1 = **0.1 < 0.2 = 0.2** < 0.6 < 1.0 vs. [0.7:1]
2) SCC: 0.2 = **0.2 = 0.2 = 0.2** < 0.6 < 1.0 vs. [0.7:1]
3) SCC: 0.2 = **0.2 < 0.3 = 0.3** < 0.6 < 1.0 vs. [0.7:1]

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

5) BD: 0.3 = **0.3 < 0.4 = 0.4** < 0.8 < 1.0 vs. [0.9:1]
6) SCC: 0.4 = **0.4 = 0.4 = 0.4** < 0.7 < 1.0 vs. [0.9:1]

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

EXAMPLE 4

BCC

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

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

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

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

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

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

EXAMPLE 5

SCC

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

3) SCC: 0.4 = **0.4 = 0.4 = 0.4** < 0.7 < 1.0 vs. [0.9:1]
4) SCC: 0.7 > **0.6 < 0.7 = 0.7** < 0.9 < 1.0 vs. [1:1]

EXAMPLE 6

MM

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

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

EXAMPLE 7

ALL MEASURED PATHOLOGIES

HS: **0.6 > 0.5 = 0.5** < 0.8 < 1.0 vs. [0.7 : 1]

BCC: **0.4 = 0.4 = 0.4** < 0.7 < 1.0 vs. [1:1]
BCC: **0.4 = 0.4 = 0.4** < 0.7 < 1.0 vs. [1:1]
BCC: **0.4 < 0.5 = 0.5** < 0.8 < 1.0 vs. [1:1]
SCC: **0.6 < 0.7 = 0.7** < 0.9 < 1.0 vs. [1:1]

SCC: **0.4 = 0.4 = 0.4** < 0.7 < 1.0 vs. [0.9:1]
BCN: **0.2 < 0.3 < 0.4** < 0.7 < 1.0 vs. [0.9:1]
BCN: **0.2 < 0.3 < 0.4** < 0.7 < 1.0 vs. [0.9:1]
BD: **0.3 < 0.4 = 0.4** < 0.8 < 1.0 vs. [0.9:1]

6) MM: **0.4 < 0.5 = 0.5** < 0.8 < 1.0 vs. [0.8:1]
7) MM: **0.3 < 0.4 = 0.4** < 0.8 < 1.0 vs. [0.8:1]
8) BCC: **0.2 < 0.3 = 0.3** < 0.7 < 1.0 vs. [0.8:1]
BCC: **0.2 < 0.3 = 0.3** < 0.7 < 1.0 vs. [0.8:1]
BCC: **0.2 < 0.3 = 0.3** <0.7 < 1.0 vs. [0.8:1]
BCN: **0.2 < 0.3 = 0.3** < 0.7 < 1.0 vs. [0.8:1]
BD: **0.2 < 0.3 = 0.3** < 0.7 < 1.0 vs. [0.8:1]

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

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

MM: **0.4 < 0.5 < 0.5** < 0.8 < 1.0 vs. [0.7:1]

BCN: **0.2 < 0.3 = 0.3** < 0.7 < 1.0 vs. [0.7:1]

BCN: **0.3 < 0.4 = 0.4** < 0.8 < 1.0 vs. [0.7:1]

BD: **0.1 < 0.2 = 0.2** < 0.6 < 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 cancers, generally indicating low, medium and high activity levels in cells in tissues.

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CLAIMS

1. **A method of common and specific characterization of developmental stages of human skin carcinogenesis in intact benign, premalignant and malignant human skin tumourous tissues based on DNA and protein pattern-recognized sequence analysis in the mid-IR spectra by FTIR microspectroscopy, the method comprising of:**

(i) evolutionary observation of expression of nucleic acids and proteins in DNA and protein sequences, thus for identification of patterns of DNA and protein sequences in benign, premalignant and malignant human skin tumourous tissues; and

(ii) tissue-specific recognition of patterns of DNA and protein sequences, thus distinctive for benign, premalignant and malignant human skin tumourous tissues; and

(iii)tumour-specific recognition of patterns of DNA and protein sequences, thus distinctive for benign, premalignant and malignant skin lesions

wherein said DNA and protein patterned sequence recognitions are always compared with patterns, specific for intact human healthy epidermal tissue.

2. **The use of Claim 1, wherein the method of recognition of tissue- and tumour-specific patterns of DNA sequences in FTIR spectra is characterized by:**

(i) DNA sequence detection by means of determination of DNA-specific frequency bands over a range of known bands at about 965 cm^{-1}, 1055 cm^{-1}, and **at about 1071 cm^{-1}, 1084/1085 cm^{-1}, 1095 cm^{-1} in the DNA/RNA triad**, to find the following **DNA sequence** patterns, as:

(a) "DNA(965), DNA/RNA triad (1071, 1084/1085, 1095)", distinctive for healthy tissue;
(b) "DNA(965), DNA/RNA(1055), DNA/RNA triad (1071, 1084/1085, 1095)", distinctive for benign, premalignant and malignant cancerous tissue;

(ii) mean value calculation of each frequency band, detected in DNA sequence as "DNA(965), DNA/RNA(1055), DNA/RNA triad (1071, 1084/1085, 1095)", to find further patterns, as:

(a) "DNA(965_{mean}) < DNA/RNA(1055_{mean}) < DNA/RNA triad (1071, 1084/1085/$1095_{max\ peak\ mean}$)", characteristic for skin benign tumours;

(b) "DNA(965_{mean}) < DNA/RNA(1055_{mean}) < DNA/RNAtriad (1071, 1084/1085/$1095_{max\ peak\ mean}$)", characteristic for skin premalignant tumours;

(c) "DNA(965_{mean}) < DNA/RNA(1055_{mean}) < DNA/RNAtriad (1071, 1084/1085/$1095_{max\ peak\ mean}$)", **and**
"DNA(965_{mean}) < DNA/RNA(1055_{mean}) > DNA/RNAtriad (1071, 1084/1085/$1095_{max\ peak}$

$_{mean)}$", **and**
"DNA(965_{mean}) > DNA/RNA(1055_{mean}) < DNA/RNAtriad (1071, 1084/1085/$1095_{max\ peak\ mean}$)",
characteristic for skin malignant tumours.

(iii) mean intensity calculation of each frequency band detected in DNA sequence to find further patterns, as:

(a) "DNA($I_{(965mean)}/I_{(1245mean)}$) < DNA/RNA($I_{(1055mean)}/I_{(1245mean)}$) < DNA/RNAtriad ($I_{(DNA/RNA\ triad\ max\ peak\ mean)}/I_{(1245mean)}$)", characteristic for skin benign tumours;
(b) "DNA($I_{(965mean)}/I_{(1245mean)}$) < DNA/RNA($I_{(1055mean)}/I_{(1245mean)}$) < DNA/RNAtriad ($I_{(DNA/RNA\ triad\ max\ peak\ mean)}/I_{(1245mean)}$)", characteristic for skin premalignant tumours;
(c) "DNA($I_{(965mean)}/I_{(1245mean)}$) < DNA/RNA($I_{(1055mean)}/I_{(1245mean)}$) < DNA/RNAtriad ($I_{(DNA/RNA\ triad\ max\ peak\ mean)}/I_{(1245mean)}$)", as well
"DNA($I_{(965mean)}/I_{(1245mean)}$) < DNA/RNA($I_{(1055mean)}/I_{(1245mean)}$) > ($I_{(DNA/RNA\ triad\ max\ peak\ mean)}/I_{(1245mean)}$)", as well
"DNA($I_{(965mean)}/I_{(1245mean)}$) > DNA/RNA($I_{(1055mean)}/I_{(1245mean)}$) < ($I_{(DNA/RNA\ triad\ max\ peak\ mean)}/I_{(1245mean)}$)",
characteristic for skin malignant tumours.

(iv) calculation of proportional ratio to characterize epidermal tissue towards skin cancer development within the following ranges, as:

(a) [0.5:1] - [0.7:1], specific for healthy epidermis;

(b) [0.7:1] - [0.9:1], specific for skin benign tumours;

(c) [0.8:1] - [0.9:1], specific for skin premalignant tumours;

(d) [0.7:1]-[1:1], specific for skin malignant tumours, BUT dependent on the type of tumour.

3. The use of Claim 1, wherein the method of recognition of tissue- and tumour-specific patterns of protein sequences in FTIR spectra is characterized by:

(i) mean value calculation of each protein-specific frequency band in detected protein sequence, for descriptive and non-descriptive proteins at about 1310 cm^{-1}, 1390 cm^{-1}, 1450 cm^{-1}, 1540 cm^{-1} and 1650 cm^{-1}, wherein

(a) expressed mean values of non-descriptive, i.e. non-specific, protein at about 1310 cm^{-1} determine the starting level, i.e. activity level, in detected protein sequences from healthy, benign, premalignant and malignant cancerous tissues
(b) mean values of non-descriptive, i.e. non-specific, protein at about 1310 cm^{-1} in protein sequences are ranged in strong correlation with gradation in proportional ratios
(c) mean values of non-descriptive, i.e. non-specific protein at about 1310 cm^{-1} in protein sequences are always at similar values expressed by activity levels of DNA/amide III peak at about 1245 cm^{-1}
(d) mean values of non-desciptive, i.e. non-specific, proteins at about 1390 cm^{-1} and 1450 cm^{-1} are expressed equally in protein sequences of premalignant and malignant cancerous tissues, but not of benign cancerous tissue vs. [0.9:1]
(e) mean values of specific proteins at about 1540 cm^{-1} are expressed in strong

correlation with gradation in proportional ratios, but differ for benign, premalignant and malignant cancerous tissues

(ii) a comparative analysis of the activity of expressed mean values in protein sequences related to benign, premalignant and malignant cancerous tissues, as:

(a) specific for benign skin tumours, with reference to benign compound nevus, characterized by:

- 2 patterns of non-specific protein sequences, as "(1310) < (1390) = (1450)" and "(1310) < (1390) < (1450)"
- the pattern, "(1310) < (1390) = (1450)", is for ranged mean values that is correlated with [0.7:1] and [0.8:1] proportional ratios
- the pattern, "(1310) < (1390) < (1450)" is for ranged mean values that is in strong correlation with [0.9:1] proportional ratio
- mean values for non-specific proteins, ranged within 0.2-0.4, and mean values for specific protein at about 1540 cm^{-1}, ranged within 0.7-0.8, do not show correlation with any of the gradation in proportional ratios between [0.7:1]-[0.9:1]

(b) specific for premalignant cancerous tissue, with reference to Bowen's disease, characterized by:

- one-patterned non-specific protein sequences, as "(1310) < (1390) = (1450)"
- this pattern is strongly correlated with gradation in proportional ratios
- mean values for non-specific proteins, ranged within 0.1-0.4, and mean values for specific protein at about 1540 cm^{-1}, ranged within 0.6-0.8, increase with gradation increment in proportional ratios from [0.7:1] to [1:1]

(c) specific for malignant cancerous tissue,

with reference to basal cell carcinoma, characterized by:

- 2 patterns of non-specific protein sequences, as "(1310) = (1390) = (1450)" and "(1310) < (1390) = (1450)"
- the pattern "(1310) = (1390) = (1450)" is only correlated with the gradation in [1:1] proportional ratios
- mean values for non-specific proteins, ranged within 0.2-0.5 in the pattern as of "(1310) < (1390) = (1450)", and mean values for specific proteins at about 1540 cm^{-1}, ranged within 0.7-0.8, are expressed in strong correlation with gradation increment in proportion ratios from [0.8:1] to [1:1]

with reference to squamous cell carcinoma, characterized by:

- 2 patterns of non-specific protein sequences, as "(1310) = (1390) = (1450)" and "(1310) < (1390) = (1450)"
- these patterns are not correlated with the gradation in proportional ratios
- high-ranged mean values for non-specific proteins within 0.2-0.7, and mean values for specific proteins at about 1540 cm^{-1} ranged within 0.6-0.9, are expressed in strong correlation with gradation increment in proportional ratios from [0.7:1] to [1:1]

with reference to malignant melanoma, characterized by:

- one-patterned non-specific protein sequences, as "(1310) < (1390) = (1450)"

- low-ranged mean values for non-specific proteins within 0.3-0.5 are expressed with mean values of 0.8 for specific proteins at about 1540 cm^{-1}, that are higher than for benign tumour

4. The use of Claim 1, wherein a method, comprised of steps in Claim 1, is to further indicate common and specific features in examined tissue towards skin cancer development by means of comparing expressed mean values and mean intensities of each frequency band, detected in DNA and protein sequences, with proportional ratios, to indicate the grade of activity in cells of benign, premalignant and malignant cancerous tissues, as well to analyse a systemic progression of the grade of activity within one type of tumour and between different types of tumour, comprising of:

(i) detection of the lowest and the highest mean values and intensities in all patterned DNA sequences specific to each kind of cancerous tissue to commonly characterize cancer progression, as:

(a) in benign cancerous tissue with reference to benign compound nevus

with the *lowest mean values*,

as "DNA(0.12) < DNA/RNA(0.17) < DNA/RNA triad(0.19) vs. [0.7:1]

and the *lowest intensities*,

as "DNA(0.48) <DNA/RNA(0.68) < DNA/RNA triad(0.76) vs. [0.7:1];

with the *highest mean values*,

as "DNA(0.18) < DNA/RNA(0.24) < DNA/RNA triad(0.27) vs. [0.9:1],

and with the *highest intensities*,

as "DNA(0.56) <DNA/RNA(0.75) < DNA/RNA triad(0.84) vs. [0.9:1].

(b) in precancerous tissue with reference to Bowen's disease

with the *lowest mean values*,

as "DNA(0.09) < DNA/RNA(0.15) < DNA/RNA triad(0.18) vs. [0.8:1],

and the *lowest intensities*,

as "DNA(0.41) <DNA/RNA(0.68) < DNA/RNA triad(0.82) vs. [0.8:1];

with the *highest mean values*,

as "DNA(0.23) < DNA/RNA(0.26) < DNA/RNA triad(0.29) vs. [0.9:1],
and with the *highest intensities*,
as "DNA(0.70) <DNA/RNA(0.79) < DNA/RNA triad(0.88) vs. [0.9:1].

(c) in malignant cancerous tissue,

with reference to squamous cell carcinoma,

with the *lowest mean values*,
as "DNA(0.19) > DNA/RNA(0.13) < DNA/RNA triad(0.16) vs. [0.7:1],
and with the *lowest intensities*,
as "DNA(0.41) < DNA/RNA(0.77) > DNA/RNA triad(0.71) vs. [0.7:1].

with the *highest mean values*,
as "DNA(0.60) < DNA/RNA(0.66) > DNA/RNA triad(0.65) vs. [1:1],
and with *the highest intensities*,
as "DNA(0.91) < DNA/RNA(1.00) > DNA/RNA triad(0.99) vs. [1:1];

with reference to basal cell carcinoma,

with the *lowest mean values*,
as "DNA(0.08) < DNA/RNA(0.14) < DNA/RNA triad(0.16) vs. [0.8:1],
and with the *lowest intensities*,
as "DNA(0.38) < DNA/RNA(0.67) < DNA/RNA triad(0.76) vs. [0.8:1];

with the *highest mean values*,
as "DNA(0.35) < DNA/RNA(0.44) < DNA/RNA triad(0.45) vs. [1:1],
and with the *highest intensities*,
as "DNA(0.75) < DNA/RNA(0.94) < DNA/RNA triad(0.96) vs. [1:1].

with reference to malignant melanoma,

with the *lowest/highest mean values*,
as "DNA(0.23) < DNA/RNA(0.26) < DNA/RNA triad(0.30) vs. [0.8:1],
as "DNA(0.19) > DNA/RNA(0.14) < DNA/RNA triad(0.27) vs. [0.8:1];

with the *lowest/highest intensities*,
as "DNA(0.61) < DNA/RNA(0.69) < DNA/RNA triad(0.79) vs. [0.8:1],
as "DNA(0.63) > DNA/RNA(0.47) < DNA/RNA triad(0.90) vs. [0.8:1].

(ii) correlating the lowest/highest expressed mean values and calculated intensities in detected DNA sequences with proportional ratios, to specifically characterize cancer progression within one type of tumour, as:

(a) in benign skin tumours, with reference to benign compound nevus, characterized by:
-one pattern of DNA sequence as "DNA(965) < DNA/RNA(1055) < DNA/RNA triad(1071, 1084/1085, 1095)"
-this pattern is independent on the grade of activity by proportional ratios
-this pattern is characterized in low-ranged mean values and calculated intensities when compared with premalignant and malignant skin tumours, that are well-correlated with gradation in proportional ratios

(b) in premalignant skin tumours, with reference to Bowen's disease, characterized by:
-one pattern of DNA sequence as "DNA(965) < DNA/RNA(1055) < DNA/RNA triad(1071, 1084/1085, 1095)"
-this pattern is independent on the grade of activity by proportional ratios
-this pattern is characterized by well-correlated with gradation in proprotional ratios intermediate ranges of mean values and calculated intensities, that are higher than for benign skin tumours, but lower than for malignant skin tumours

(c) in malignant skin tumours,

with reference to basal cell carcinoma, characterized by:
-2 patterns of DNA sequences: 1) "DNA(965) < DNA/RNA(1055) < DNA/RNA triad(1071, 1084/1085, 1095)"; 2) "DNA(965) < DNA/RNA(1055) > DNA/RNA

triad(1071, 1084/1085, 1095)"
-DNA sequence pattern "DNA(965) < DNA/RNA(1055) < DNA/RNA triad (1071, 1084/1085, 1095)" is dominant, independent whether based on the expressed mean values or calculated intensities
-these patterns are independent on the grade of activity by proportional ratios
-these patterns are characterized in high-ranged mean values and calculated intensities that are well-correlated with gradation in proportional ratios, when compared with benign and premalignant skin tumours

with reference to squamous cell carcinoma, characterized by:
-3 patterns of DNA sequences as "DNA(965) < DNA(1055) > DNA/RNA triad", "DNA(965) < DNA(1055) < DNA/RNA triad" and "DNA(965) > DNA(1055) < DNA/RNA triad", based on expressed mean values, that are dependent on the grade of activity by proportional ratios and the acitivity of DNA/RNA peak at about 1055 cm^{-1},
-one-patterned DNA sequence "DNA(965) < DNA(1055) > DNA/RNA triad", based on calculated intensities, that are dependent on the grade of activity by proportional ratios and the acitivity of DNA/RNA peak at about 1055 cm^{-1}
-all patterns, independent whether based on expressed mean values or calculated intensities, are characterized by high ranges, that are higher in comparison to other malignant skin tumours, and are well-correlated with gradation in proportional ratios

with reference to malignant melanoma, characterized by:
-2 patterns of DNA sequences as "DNA(965) < DNA(1055) < DNA/RNA triad" and "DNA(965) > DNA(1055) < DNA/RNA triad", independent whether based on the expressed mean values or calculated intensities
-these patterns are independent on the grade of activity by proportional ratios
-these patterns are characterized by the highest levels of expressed mean values and calculated intensities vs. lower gradation in proportional ratios, when compared with the other types of malignant skin tumours

(iii) comparative protein sequence analysis for determination of systematic progression of the grade of activity between benign, premalignant and malignant skin tissue cells, as:

(a) specific to indicate a progression from premalignant to malignant cancerous tissue against healthy epidermal tissue,
characterized in protein sequences by expressed mean values in relation to gradation in proportional ratios, as :

with reference to healthy epidermal skin, i.e. HS, Bowen's disease (*in situ* cancer), i.e. BD, vs. squamous cell carcinoma, i.e. **SCC**

- in relation to [0.7:1]
BD: 0.1 < 0.2 = 0.2 < 0.6 < 1.0
SCC: 0.2 = 0.2 = 0.2 < 0.6 < 1.0
SCC: 0.2 < 0.3 = 0.3 < 0.6 < 1.0
HS: 0.4 = 0.4 = 0.4 = 0.4 < 0.8 < 1.0
HS: 0.5 < 0.6 > 0.5 =0.5 < 0.8 < 1.0

-in relation to [0.9:1]
BD: 0.3 <0.4 = 0.4 < 0.8 < 1.0
SCC: 0.4 = 0.4 = 0.4 < 0.7 < 1.0

(b) specific to indicate a progression from benign to malignant cancerous tissue in known pathologies to transform from benign to malignant skin tumours against healthy epidermal tissue,
characterized in protein sequences by expressed mean values in relation to gradation in proportional ratios, as:

with reference to healthy epidermal skin, i.e. HS, benign compound nevus, i.e. BCN vs. malignant melanoma (MM)

- in relation to [0.7:1]:
BCN: 0.3 < 0.4 = 0.4 < 0.8 < 1.0
BCN: 0.2 < 0.3 = 0.3 < 0.7 < 1.0
MM: 0.4 < 0.5 = 0.5 < 0.8 < 1.0
HS: 0.4 = 0.4 = 0.4 = 0.4 < 0.8 < 1.0

HS: 0.5 < 0.6 > 0.5 =0.5 < 0.8 < 1.0

- in relation to [0.8:1]
BCN: 0.2 < 0.3 = 0.3 < 0.7 < 1.0
MM: 0.4 <0.5 = 0.5 < 0.8 < 1.0
MM: 0.3 < 0.4 = 0.4 < 0.8 < 1.0

(c) specific to indicate a progression from benign to premalignant, from premalignant to malignant skin cancerous tissue, against healthy epidermal tissue, characterized in protein sequences by expressed mean values in relation to gradation in proportional ratios, as:

-in relation to [0.7:1]
BCN: 0.2 < 0.3 = 0.3 < 0.7 < 1.0
BCN: 0.3 < 0.4 = 0.4 < 0.8 < 1.0
BD: 0.1 < 0.2 = 0.2 < 0.6 < 1.0
SCC: 0.2 = 0.2 = 0.2 < 0.6 < 1.0
SCC: 0.2 < 0.3 = 0.3 < 0.6 < 1.0
MM: 0.4 < 0.5 = 0.5 < 0.8 < 1.0
HS: 0.4 = 0.4 = 0.4 = 0.4 < 0.8 < 1.0
HS: 0.5 < 0.6 > 0.5 =0.5 < 0.8 < 1.0

in relation to [0.8:1]
BCN: 0.2 < 0.3 = 0.3 < 0.7 < 1.0
BD: 0.2 < 0.3 = 0.3 < 0.7 < 1.0
BCC: 0.2 < 0.3 = 0.3 < 0.7 < 1.0
MM: 0.3 < 0.4 = 0.4 < 0.8 < 1.0
MM: 0.4 < 0.5 = 0.5 < 0.8 < 1.0

in relation to [0.9:1]
BCN: 0.2 < 0.3 < 0.4 < 0.7 < 1.0
BD: 0.3 < 0.4 = 0.4 < 0.8 < 1.0
SCC: 0.4 = 0.4 = 0.4 < 0.7 < 1.0

in relation to [1:1]

BCC: 0.4 = 0.4 = 0.4 < 0.7 < 1.0

BCC: 0.4 = 0.4 = 0.4 < 0.8 < 1.0

BCC: 0.4 < 0.5 = 0.5 < 0.8 < 1.0

SCC: 0.6 < 0.7 = 0.7 < 0.9 < 1.0

5. The method of Claims 1, 2 and 4, wherein DNA sequence comprises a range of frequency bands at ~ 965, 1055, 1071, 1084/1085, 1095 cm-1, specific for nucleic acids in the FTIR spectra.

6. The method of Claims 1, 3 and 4, wherein protein sequence comprises a range of frequency bands at ~ 1310, 1390, 1450, 1540, 1650 cm-1, specific for proteins in the FTIR spectra.

7. The method of Claim 2, wherein said proportional ratio is a sum mean DNA/RNA triad peaks at about 1071, 1084/1085, 1095 cm^{-1} *versus* DNA/amide III peak at about 1245 cm^{-1}

8. The method of Claims 1 to 4, wherein the reference pattern is the pattern, specific for human healthy epidermal tissue, that is comprises of DNA sequence with mean values as of *0.20 (965) > 0.00 (1055) < 0.32 (1071, 1084/1085, 1095)*, and that is comprises of protein sequence with mean values as of *0.5 (1310) > 0.4 (1390) < 0.5 (1450) < 0.8 (1540) < 1.0 (1650).*

9. The method of Claims 1 to 4, wherein the pattern specific for human healthy epidermal tissue is a pattern of a healthy subject, a subject that is otherwise free of skin cancer, or a subject is free from any other skin lesion.

10. The method of Claims 1, 2 and 4, wherein said the intensities of all determined nucleic acids peaks in the 900-1300 cm-1 region, that are the peaks at ~ 965, 1055, 1071, 1084/1085, 1095 cm-1, are compared with the intensity of the peak at about 1245 cm-1 by calculating the following intensity peaks ratios: I(965)/I(1245), I(1055)/I(1245), I(max peak among DNA/RNA triad peaks)/I(1245).

11. The method of Claims 1, 3 and 4, wherein said the intensities of all determined peaks for specific and nonspecific proteins in the 1300-1700 cm-1 region, that are the peaks at ~ 1310, 1390, 1450, 1540, 1650 cm-1, compared with the intensity of amide I peak at about 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).

12. The method of Claims 1 to 4, wherein the detecting step is performed by **Fourier transform** infrared spectrometer and/or **Fourier tranform** infrared microspectrometer.

13. The method of Claims 1 to 4, wherein said common and specific characterization of developmental stages of skin carcinogenesis in intact benign, premalignant and malignant human skin tumourous tissues based on DNA and protein patterned recognized sequence analysis in the mid-IR spectra by FTIR microspectroscopy, that is also applicable to **intact** animal tissue.

14. The method of Claims 1 to 4, wherein said intact benign, premalignant and malignant human skin tumourous tissues, that is also applicable to tissue from other organs.