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