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Melanoma Volatile Fingerprint with a Gas Sensor Array:

In Vivo and In Vitro Study

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Abstract

Volatile compounds fingerprinting with gas sensor arrays is a promising non-invasive methodology for human health-state monitoring. Anyway, the large variability of human samples (breath, urine, sweat, skin transpiration) may have a complex influence in the composition of the volatile part and in the reproducibility of the analyses. Nonetheless, skin analysis is a favourable application where each subject can provide its own reference: free skin portions compared with skin lesions, avoiding the masking effects of skin volatiles composition variability. Moreover, in vitro measurements of melanoma cell lines show that enough information to recognize different tumor-types can be obtained by cells headspace.

Keywords: Volatile compounds fingerprinting; Gas sensor array; Non-invasive diagnosis; Melanoma; Cell headspace

1. Introduction

When a nevus undergoes metabolic changes generated by melanocytic cells it becomes a melanoma. Surgery seems to be the only efficient therapy for melanoma. However it is only considered to be a complete cure when accompanied by an early diagnosis since both chemo and radiotherapy are ineffective in advanced and metastatic melanoma.

Over the last few years the simple technique of lesion inspection employed by dermatologists has been gradually fortified with the adoption of non-invasive imaging based methods[1]: dermatoscopy is the most simple of these methods, the more sophisticated method is the digitally based epiluminescence imaging, while confocal laser

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microscopy is the more recently introduced diagnostic technique. On this basis, we can say that none of these methodologies can provide a 'skin lesion fingerprint' that can be univocally identified as a nevus or melanoma 'signature'. Actually the reliability of visual lesion inspection by dermatologists is only considered to be 70% which would suggest that the visible features of the remaining 30% are unclear.

The cited methodologies, otherwise, are often based on traditional imaging techniques rather than on 'chemical imaging'. The thesis of this paper is to demonstrate that the alteration of the 'internal chemistry' when a nevus turns into a melanoma alters the pattern of volatile compounds emitted by the nevus itself. This thesis is supported by the recent study by Gallagher et al. on the characterization of volatile compounds emitted by skin[2], and by the possibility to identify melanoma through airborne chemicals, as shown by trained dogs that uses their fine olfaction to locate tumours[3]. Herewith, we present experiments aimed at detecting, with an electronic nose, in vivo melanoma tissues[4] and in vitro melanoma cells.

2. Experimental

2.1 Gas Sensor array

In this work the last version of the gas sensors array developed at the University of Rome 'Tor Vergata' was used. The same instrument has been also positively applied in many other studies in medicine[5]. The measure cell of the instrument contains an array of seven quartz micro balance (QMB) chemical sensors, each coated with a different metalloporphyrin[6].

QMB sensors working principle is based on the fact that the resonant frequency of a quartz crystal covered by a sensitive material is proportional to the quantity of mass absorbed by the sensitive layer during the exposure of the sensors to the sample; thus, these sensors allow measurements of the amount of molecules absorbed from the gas phase onto the sensing layer[7].

The response pattern of the electronic nose sensors is formed by the steady-state frequency shifts registered; the set of patterns obtained from series of measurements can be properly analysed by some pattern-recognition algorithm for classification purposes.

2.2 Sampling Procedure

The design of the procedure for sample uptake is of course decisive but often underestimated; actually, an optimal sampling methodology is necessary to guarantee measurements reproducibility and samples representativity. A stainless-steel cylinder (diameters: 4 cm) was used in vivo measurements to insulate a skin region The area of all the skin lesions investigated fitted with the size of the sampler used. Previous investigations[8] put in evidence a large subjective variability of chemicals emitted by skin in terms of quantity and quality; in order to face this problem, a differential measurement strategy[4] was adopted.

Another stainless-steel cylinder (diameter: 6 cm) was used for in vitro measurements to fit the Petri plate containing the cells.

2.3 In vivo measurements

In-vivo experiment[4], carried out at the 'Istituto Dermopatico dell'Immacolata' (Rome, Italy) involved 40 subjects presenting suspected skin lesions, 10 of them were melanomas as confirmed by post-surgery histology. Epiluminescence examination was performed for each lesion that was measured with the gas sensor array.

2.4 In vitro measurements

In vitro experiments were carried out at the Department of Pathology of the St. Andrea Hospital in Rome, on five tumoral cells lines: three melanoma cancers, a synovial sarcoma and a thyroid cancer. Cells were derived from the respective primary human tumors and cultured in standard conditions in Petri dishes with RPMI-1640 medium supplemented with 2 mM glutamine, 10% Fetal calf serum, penicillin and streptomycin (GIBCO BRL, Gaithersburg, MD) at 37°C and 5% CO_2 atmosphere.

3. Results and conclusions

Multivariate analysis is a natural approach for such data; on the other hand a preliminary study of the behaviour of each sensor, is useful to understand the potentialities of the instrument. A significant test of this preliminary data treatment is the comparison of the patterns related to the same patient in the same skin location, but taken before and after the surgical treatment. Seven of the 10 Melanoma cases was analyzed 15 days after surgery when also the histological report was available.

Two examples are reported in Fig. 1 (patient 1 and patient 2): it is evident the large reduction of the response registered for each sensor after the surgical removal of the melanoma.
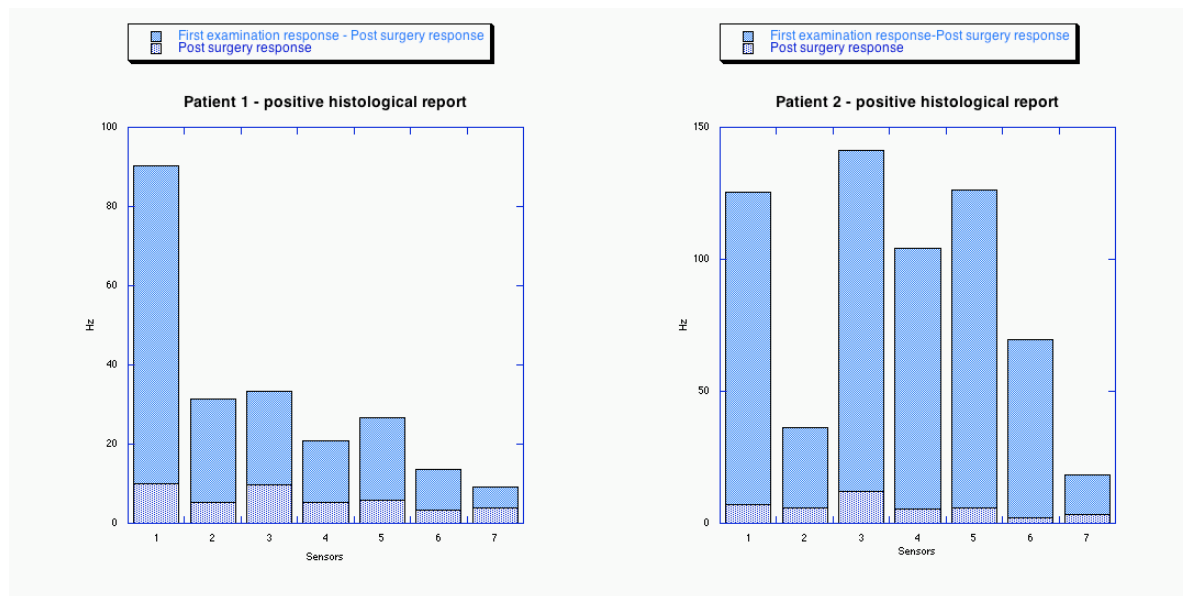

Fig. 1. Sensors response pattern related to the same individual (patient 1 (left), patient 2 (right)) before and after the surgical removal of skin lesion. The decreasing of the signals registered after the removal of the melanoma is evident for each sensor and for both the patients. Histological report confirmed the diagnosis of melanoma.

A PLS-DA model aimed at classifying nevi from melanomas was built in matlab. The model was properly optimized by the leave-one-out cross-validation method. The total percentage of correct classification in prediction was 87% ; it is largely comparable with other diagnosis methods currently in use for melanoma.

This good performance is affected by the bias of a 1:4 ratio for the populations of the two classes (nevi and melanomas) resulting in a sensitivity of 70% and a specificity of 90%.

The score plot of the first two latent variables of the PLS-DA model is reported in Fig. 2.a, which shows the separation between the distribution of nevi and melanoma. A small number of melanoma cases fall in the nevi region and vice versa. The identification of melanoma by volatile compounds analysis was firstly suggested by experiences with trained dogs. Nonetheless, because of the complexity of dogs' perception the interpretation of these experiments is still debated and they cannot be considered as a definitive demonstration of the existence of univocal correlation between disease and the volatile compounds profile. The possibility to realize a differential measurement strategy is a favourable experimental condition for the electronic nose; actually, melanoma is a very localized alteration that can be insulated and measured with respect to a close unaltered skin region

In spite of a good strategy for skin volatile compounds measurement, the evidences obtained in the first part of the experiment still in progress, are obviously partial respect to a series of further aspects needing to be clarified. A

decisive point is to understand if the volatile compounds which allowed to discriminate between the positive and the negative samples are originated by the tumor or they are the results of a general alteration of the individual health-state due to the presence of the tumor itself. The in vitro experiment seems to provide preliminary indication about some tumor-specific volatile markers (not identified in this work). Actually, Results indicate that the same gas sensors array can capture the differences between melanoma cells with respect to other tumors cells by the analysis of the volatiles in the cells headspace (as it can be observed from the Principal Component Analysis scores plot reported in figure 2.b).
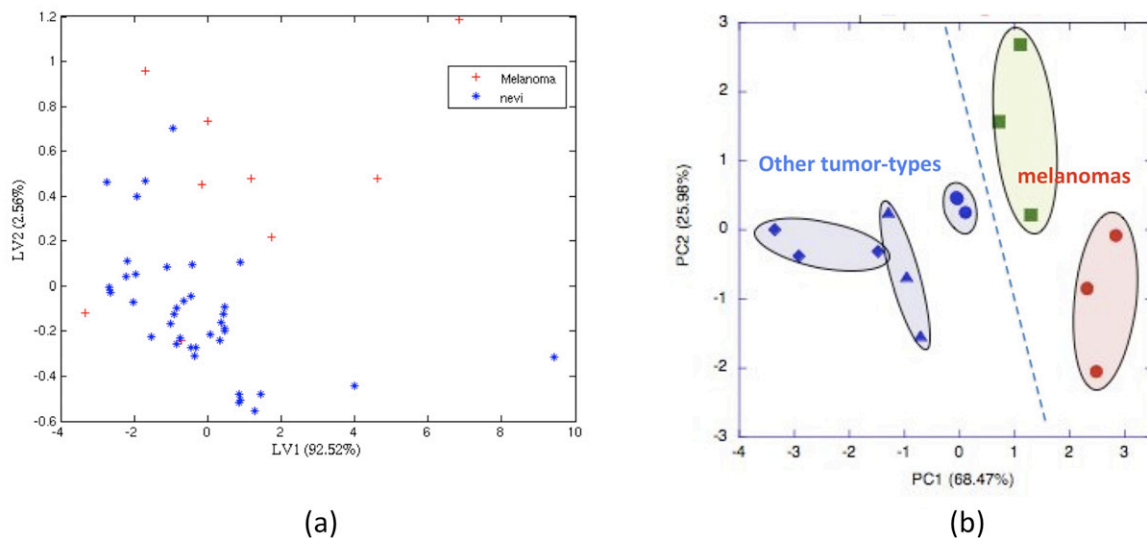

Fig. 2. (a) Scores Plot of the first two LVs of the PLS model built on the data collected from in vivo control and melanoma skin lesions. A good discrimination between the two groups is visible, and it is confirmed by a correct classification percentage of 87% ; (b) Scores Plot of the first two PCs of the PCA model built on the cell cultures headspace measurements. It can be observed a good discrimination between melanomas and other tumors along the PC1 (68.47% of the total variance).

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