Fluorescence Studies on Lung Tumors

Wolfgang Lohmann[1], Burkhard Hirzinger[1], Juliane Braun[1], Konrad Schwemmle[2], Karl-Heinz Muhrer[2], and Andreas Schulz[3]

Departments of [1]Biophysics, [2]Surgery and [3]Pathology, Justus-Liebig-University, Leihgesterner Weg 217, D-6300 Gießen, Bundesrepublik Deutschland

Z. Naturforsch. **45c,** 1063–1066 (1990); received April 6, 1990

Native Fluorescence, Tumor, Lung

Illumination of unstained 9 μm cryosections of lung tissue with 365 nm results in visible fluorescence light with a maximum intensity at about 460 nm. These fluorescence tomographical studies can be used for detecting carcinoma of the lung. The fluorescence pattern obtained can be matched nicely with histological findings. Since it takes less than 5 min for getting the fluorescence images, the fluorescence tomographical technique might be used in addition to established methods for determining the histology of a biopsy sample.

Introduction

Recently we have shown that a non-invasive, non-destructive fluorescence technique, which is based on naturally occurring fluorophore(s), can be used in situ measurements for an early detection of malignant melanomas and for their distinction with nevi. According to this method, healthy skin exhibits fluorescence light with a maximum at about 460 nm (after lamp intensity correction) when excited with monochromatic light at 365 nm [1]. In the case of a malignant melanoma, the fluorescence intensity is, however, very low within the tumor region and up to six times higher in the environment of the tumor than in normal healthy skin (50–100 counts/100 ms).

These results have been confirmed histologically and by the determination of the fluorophore distribution within the corresponding tissue by using unstained cryo-sections [2].

Similar results were also obtained with excised samples of the cervix uteri [3, 4] which could be confirmed more recently by Svanberg *et al.* [5] for brain tumor and breast cancer. Therefore, it seems to be obvious that the fluorescence technique might also be applicable to other types of tissue, such as lung.

Materials and Methods

Fluorescence spectra of excised tissue samples of lung were recorded right after surgery. Thereafter, a sequence of 6 successive cryo-sections (9 μm each) was prepared; each alternating one was used for fluorescence microscopy or for histological examination, after HE staining. Details of the technique used have been published elsewhere [1, 4].

The fluorescence and the histological investigations as well as the photodocumentation were done with a Zeiss Axiophot microscope.

A total of 30 patients, 34 to 80 years old and of both sexes (25 males, 5 females), with cancer of the lung has been investigated. As controls unaffected samples from these patients (as verified by histology) and from two healthy persons were used.

Since the results obtained are very similar within each category used (healthy, malignant), only one representative example is shown.

Results und Discussion

The fluorescence spectra (not shown) obtained after excitation of the excised lung samples with 365 nm are very similar to the ones reported for other types of tissue. Here, too, a healthy lung exhibits about 50–100 counts/100 ms. In the tumor region there is almost no fluorescence at all, while the highest intensity can be detected in the surrounding of the tumor. Because of the limited spatial resolution of the fluorescence spectrometer used, a more accurate intensity distribution can be obtained only with the tomographical fluorescence technique (2 D imaging) using 9 μm cryosections.

The fluorescence pattern of healthy lung tissue with bronchi located far away from cancer tissue of a 61 year old male is shown in Fig. 1 a. The right lung of this patient was removed because of poorly

Reprint requests to Prof. Dr. W. Lohmann.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/90/0900–1063 $ 01.30/0

differentiated squamous cell carcinoma. The elastic fibers of the bronchial walls can be seen very clearly. The remaining tissue exhibits the fluorescence density usually observed in healthy tissue. The high fluorescence intensity of the elastic fibers may be caused by an enlarged NADH concentration stimulated by either a high oxygen tension being present within the lung or the influence of the distant tumor. For comparison, the HE stained image is shown in Fig. 1 b. Both images match nicely.

The double wall structure of the elastic fibers of a pulmonary arterial branch (44 year old male; poorly differentiated squamous cell carcinoma) is even better documented in Fig. 2. The internal and

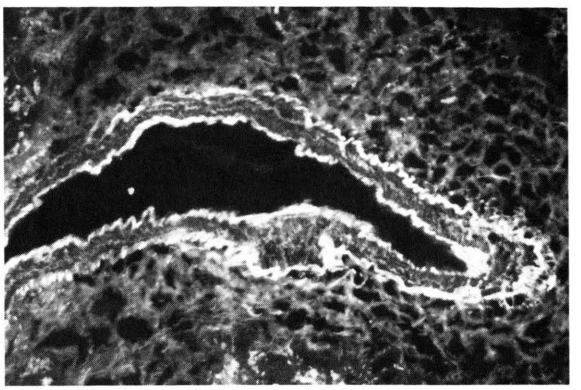
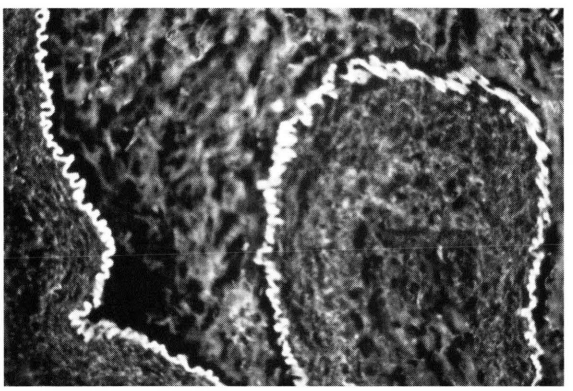


Fig. 2. Fluorescence image of an unstained 9 µm cryosection of healthy lung tissue with bronchi. Magnification 100 ×.

a



b

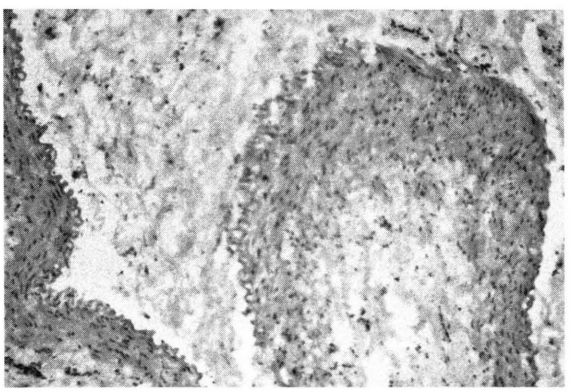
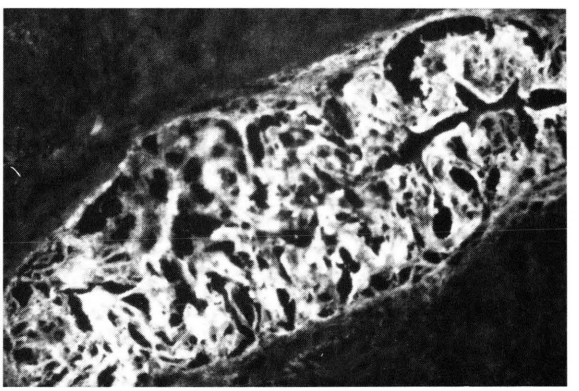


Fig. 1. Fluorescence image (a) of an unstained 9 µm cryosection of healthy lung tissue with bronchi compared with an HE-stained section of the same region (b). Magnification 100 ×.

a



b

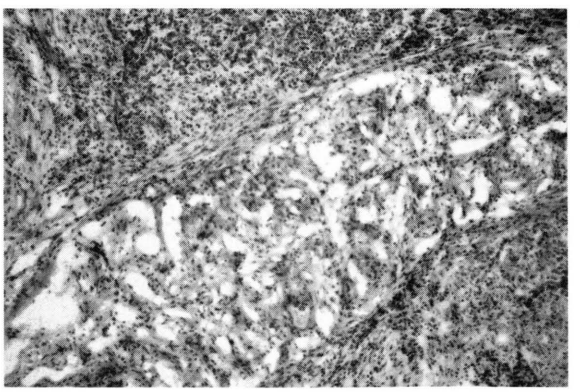
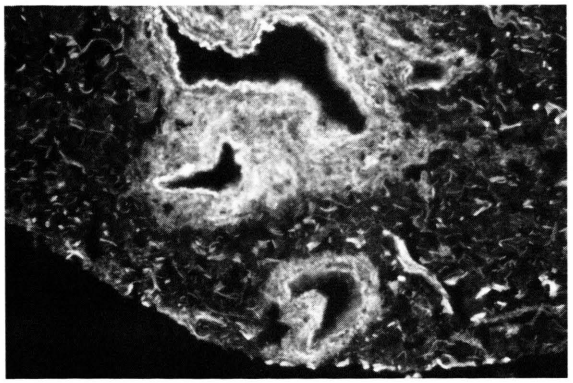


Fig. 3. Fluorescence image (a) of an unstained 9 µm cryosection of lung tissue with cancer (poorly differentiated squamous cell carcinoma) compared with an HE-stained section of the same region (b). Magnification 100 ×.

a



b

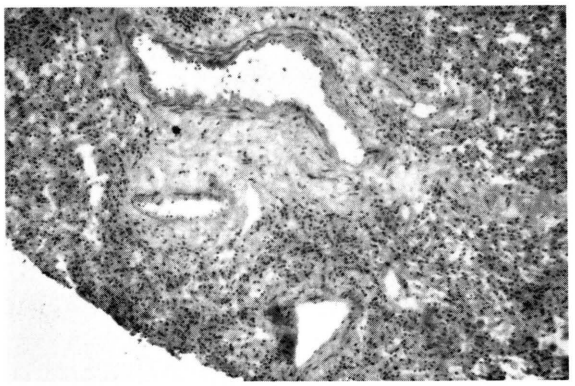
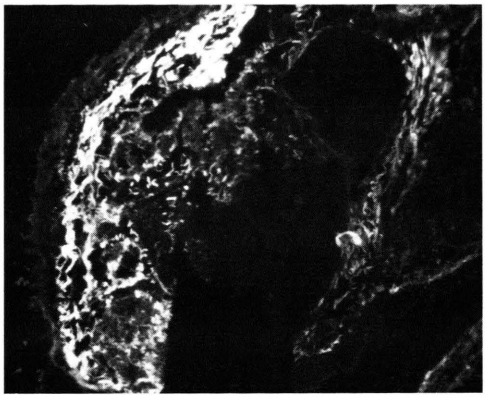


Fig. 4. Fluorescence image (a) of an unstained 9 µm cryosection of healthy lung tissue with bronchi and vessels located about 2 cm away from a tumor (shown in Fig. 3) compared with an HE-stained section of the same region (b). Magnification 100 ×.

a



b

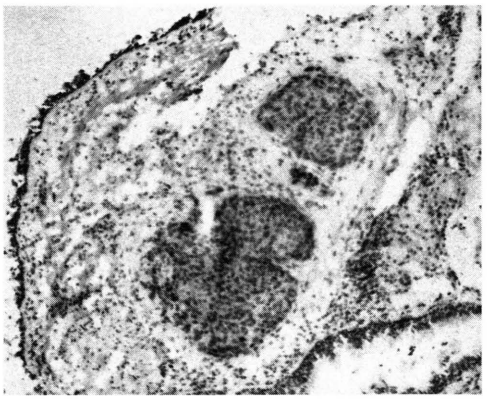


Fig. 5. Fluorescence image (a) of an unstained 9 µm cryosection of lung tissue with cancer (poorly differentiated squamous cell carcinoma) compared with an HE-stained section of the same region (b). Magnification 100 ×.

external elastic laminae are clearly shown. Again, the surrounding tissue exhibits no obvious modifications as judged by the fluorescence pattern.

In the case of bronchial cancer (56 year old male), the tumor area does not show any fluorescence at all (compare Fig. 3a and b). The area not infiltrated by the tumor, exhibits a rather high fluorescence intensity (Fig. 3a).

It is interesting to note, that in all cases investigated microscopically thus far, the immediate surrounding of bronchi, vessels or lung parenchyma, which are located near a tumor, exhibit a very high fluorescence intensity (s. Fig. 4a). Even the area which is not in the immediate environment of the bronchi or vessels shows elastic fibers with greater volume than usual and higher fluorescence intensity. This might indicate that apparently healthy tissue is already affected on a molecular level, an effect which can be detected at present only by the fluorescence technique. The histological image is shown in Fig. 4b. The lymphocytes seen there are without any influence on the fluorescence pattern.

Finally, another case (61 year old male) of pulmonary cancer (lymph vessels of the submucosa filled with tumor tissue) is shown (Fig. 5a, b). Again, the area occupied by cancer cells is dark, while the healthy surrounding area exhibits a rather high fluorescence intensity.

Acknowledgements

Part of the work was supported by the Bundesministerium für Forschung und Technologie, the Deutsche Forschungsgemeinschaft, the Fond der Chemischen Industrie and by Carl Zeiss, Co., Oberkochen. This paper is part of the MS thesis of B. H. and of the MD thesis of J. B.

[1] W. Lohmann and E. Paul, Naturwissenschaften **75,** 201–204 (1988).
[2] W. Lohmann and E. Paul, Naturwissenschaften **76,** 424–426 (1989).
[3] W. Lohmann, J. Mussmann, C. Lohmann, and W. Künzel, European Journal of Obstetrics & Gynecology and Reproductive Biology **31,** 249–253 (1989).
[4] W. Lohmann, J. Mussmann, C. Lohmann, and W. Künzel, Naturwissenschaften **76,** 125–127 (1989).
[5] St. Andersson-Engels, J. Johansson, S. Svanberg, and K. Svanberg, Analytic. Chemistry **62,** 19A–27A (1990).