Molecular Electron Paramagnetic Resonance Imaging of Melanin in Melanomas: a Proof-of-concept.

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Introduction and Objectives of the study

The incidence of malignant melanoma is increasing at alarming rates. Prevention, early detection, appropriate clinical and histological diagnoses are critical to favourable outcomes. If a lesion is suspicious for melanoma, adequate biopsy is necessary for staging and management. Thicker melanomas show greater risk of metastatic disease. In case of suspicious melanoma, there is a need to conduct a sentinel lymph node biopsy technique. Finally, the early detection of metastasis (in liver or lungs) may improve the long-term survival of patients. Newer diagnostic techniques should be evaluated, as they may reduce the need for biopsies, and may help in the early detection of distant melanoma metastases.

Our work is dealing with the development of new methods allowing the selective high resolution imaging of melanomas. Melanins are amorphous, irregular, polymeric pigments that contain organic free radicals of semiquinone type (1). This stable free radical can be easily detected at room temperature using Electron Paramagnetic Resonance (EPR) spectroscopy (2, 3). We hypothesized that the most recent developments in EPR imaging could make this technique a suitable method to map these free radicals with high sensitivity and high resolution, and render 3D-images of these malignant tissues.

Methods and Materials

Melanoma B16 implanted in C57Bl6 mice and grown subcutaneously were surgically removed and freeze-dried along with metastatic lungs. A variety of spatial and spectral-spatial (2D and 3D) EPR images have been performed on whole intact melanoma samples and metastatic lungs using a X band system.

To assess the relevance of our technique for human melanomas, we applied the method to paraffin-embedded human melanomas excised from patients.

To exclude a possible contribution of freeze-drying or histological process induced radicals, EPRI was also applied *ex vivo* and *in vivo*. *In vivo* EPR techniques (4), 1.1 GHz, using advanced surface and/or whole body coils were used to provide a signal from melanin and for imaging of melanoma.

Results

Melanin complexes in melanomas present at room temperature an EPR signal with a g value of 2.005, and a line width of around 9-10 Gauss (not shown). 2D and 3D EPR imaging give us unprecedented quantitative and qualitative information concerning the spatial distribution of paramagnetic melanin radicals in tumours or metastatic lung. The lung melanoma metastases, seen as brown spots of melanin pigments (Fig. 1a.), can be visualised by 3D EPR images (Fig. 1b.). As an illustrative example we present in Fig. 2a. human melanoma metastasis, with melanocytes irregular clusters. It can be observed that the EPR image (Fig. 2b.) is comparable with the histological section.

For the first time we were able to detect and image *in vivo* these endogenous radicals of mice SC melanoma (Fig. 3a.). The EPR spectrum was unambiguously ascribed to melanin (Fig. 3b.) and using an appropriate field gradient we could record *in vivo* an EPR 2D image of subcutaneous implanted melanoma (Fig. 3c.).

Conclusions and Perspectives

EPR imaging can provide unique information concerning the spatial distribution of the free radicals (in special melanin complexes) in melanoma and melanoma metastases. Developments in EPRI represents may open a new way for the early diagnostic of melanomas.

References

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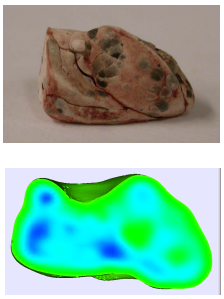

Fig.1.: top: picture of metastasized lung. Bottom: corresponding 3D EPR image showing metastasis distribution.

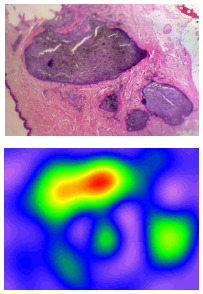

Fig.2.: top: histological section of human melanoma metastasis. Bottom: corresponding 2D EPR image showing the metastatic clusters.

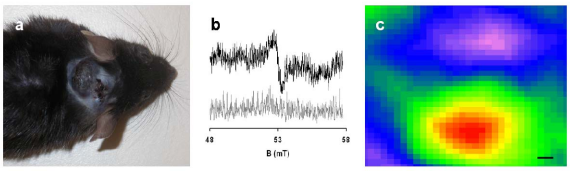

Fig.3.: a: picture of B16 melanoma in C57Bl6 mice.
b: Corresponding spectrum obtained with L-band spectrometer (black); grey spectrum shows mice without melanoma.
c: corresponding *in vivo* 2D image of subcutaneous melanoma.