Electron paramagnetic resonance spectrometry and imaging in melanomas: A comparison between pigmented and non-pigmented human malignant melanomas.

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Target audience:

EPR/EPRI scientists, MR scientists involved in cancer research and/or research on melanomas

Purpose:

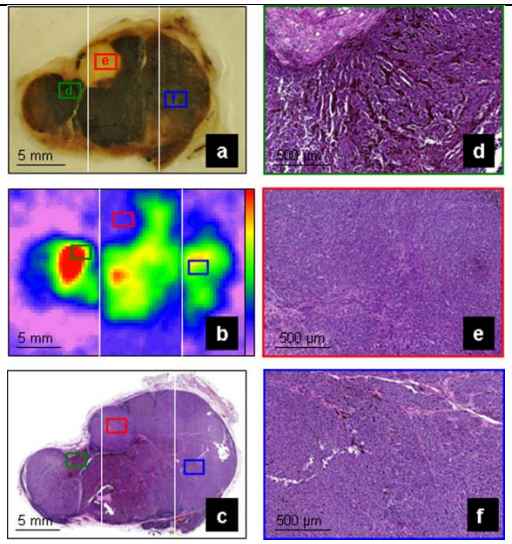
It is known for a long time that the melanin pigments present in normal skin, hair and most of malignant melanomas (1-3) can be detected by electron paramagnetic resonance (EPR) spectrometry. In this study, we proposed to use EPR imaging as a tool to map the concentration of melanin inside *ex-vivo* human pigmented and non-pigmented melanomas and to correlate this cartography with anatomo-pathology.

Methods:

Six paraffin-embedded human melanoma samples were analyzed. The samples were classified as highly pigmented (n = 3) or non-pigmented (n = 3) on the basis of the histological findings. A 500-µm-thick slice was cut from each melanoma. Each of the six slices was then cut into 2 or 3 parts due to the small size of the X-Band EPR cavity used for the EPR Imaging measurement. All spectra and images were recorded at room temperature on a Bruker E540 Elexsys system (Bruker Biospin, GmBh) equipped with a Super High Sensitivity Probe (10mm diameter, 30 mm long) operating in X-band mode at approximately 9.5 GHz and 100 KHz modulation frequency. For images, the following parameters were used: microwave power: 3.2 mW; modulation amplitude: 0.25 mT; conversion time: 10.24 ms; time constant: 5.12 ms; field of view: 23 mm; sweep width: 182 G; number of points: 1024; number of scans: 5; sweep time: 10.49 s; gradient field: 450 mT/m; number of projections: 29; pixel size: 0.6 mm; total acquisition time: 32.5 min. For each sample, a 5-µm-thick section, contiguous to the 500-µm-thick used for EPR, was processed for histology (hematoxylin-eosin staining). The histological sections were then scanned with a Zeiss Mirax microscope.

Results:

Globally, each part of the pigmented samples allowed the successful acquisition of an EPR image. All the EPR images closely reflected the shape and size of their respective sample. Accurate measurements of the dimensions showed a mean difference of 1.15% between the samples and their respective images. For the three samples, the EPR intensity in a given area was unambiguously correlated to the presence or absence of melanin pigments. To illustrate this observation, three different areas were selected on one sample P1 on the basis of the concentration of melanin visible on the histological section. A highly pigmented area was found on the left part of the sample and is presented on figure 1.d. A totally non pigmented area was found in the upper side of the middle part and is presented on figure 1.e. Finally, a moderately pigmented area was taken from the right part of the sample and is presented on figure 1F. On the EPR image, these three areas were linked to three areas with highly different intensities, the non pigmented area being impossible to distinguish from the background and the highly pigmented area exhibiting the strongest intensity. In opposition, no EPR signal coming from melanin was observed from non pigmented melanomas, demonstrating therefore the absence of EPR detectable pigments inside these particular cases of skin cancer and the importance of the pigmentation for further EPR imaging studies on melanoma.



The 500 µm-thick slice of sample P1 was cut into three parts following the white lines drawn on the figure (1.a). The three parts were measured by EPR imaging and the images were joined to reconstruct the total image of the sample (1.b). A 5 µm-thick histological section adjoining to the sample was cut (1.c) and used to correlate the pigmentation to the EPR intensity, especially on three selected areas: A highly pigmented area (1.d), a non-pigmented area (1.e), and a moderately pigmented area (1.f).

Conclusion:

The importance of the pigmentation for EPR measurement was unambiguously demonstrated. Moreover, EPR images accurately reflected the distribution of melanin pigments within melanoma samples.

References:

1) E. Vanea et al, *NMR Biomed* 2008;21:296-300; 2) Q. Godechal et al, *Contrast Media Mol Imaging* 2011;6:282-288; 3) Q. Godechal et al, *J. Skin Cancer* 2011, 2011:273280; 4) Q. Godechal et al, *Exp Dermatol* 2012;21:341-346.