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Reflectance confocal microscopy (RCM) and melanocyte-specific immunostaining of histologic skin sections

To the Editor: In vivo reflectance confocal microscopy (RCM) represents a new imaging technique that offers the possibility to examine noninvasively superficial layers of the skin at a cellular resolution.[1] The main endogenous contrast agents on RCM are melanin (refractive index $n = 1.72$) and keratin ($n = 1.51$).[2,3]

When examining the skin with in vivo RCM, it is difficult to differentiate between keratinocytes and melanocytes. With the aim of analyzing reflection pattern of the melanocyte through in vivo RCM, ex vivo RCM tests and immunohistochemistry tests were performed on identical histologic sections of skin and cutaneous melanocytic tumors for clear identification of specific cells.

It is generally assumed that melanocytes are identified as bright (hyperreflective) cells on RCM images. In this case series study, the reflectivity of melanocytes was related with histologic skin sections by applying the ex vivo RCM method as a model of clinical in vivo RCM diagnostics. Tissues were analyzed using the methods of ex vivo RCM (C1si, Nikon, Tokyo, Japan) and immunohistochemistry staining with melanocytic markers: Melan-A and HMB 45. Three skin samples (normal-appearing, Spitz nevus, and cutaneous melanoma) were obtained from 3 patients who participated in the clinical study carried out at the National Cancer Institute.[4] The study was approved by the local ethics committee (protocol number ADT-01) and performed in accordance with the Declaration of Helsinki.

As can be seen in Fig 1, *A*, melanocytic nevus cells (nevocytes) appear darker than the surrounding keratinocytes and they contain reflective nuclei, whereas the contrast of cytoplasm is blurred. Cells with this reflectance pattern are HMB 45 positive. (Fig 1, *B*). Sparce intraepidermal melanocytes (marked by arrows) are hyporefractile when examined by RCM, compared with surrounding keratinocytes with refractile cytoplasm. Similarly in melanoma samples, the majority of melanocytes (melanoma cells) are hyporefractile or isorefractile compared with surrounding keratinocytes.

The results show that melanocytes have different cytoplasm and nucleus reflectivity properties under RCM, depending on melanosome maturity: melanin-containing melanocytes have hyperreflective cytoplasm and hyporeflective nucleus; meanwhile,

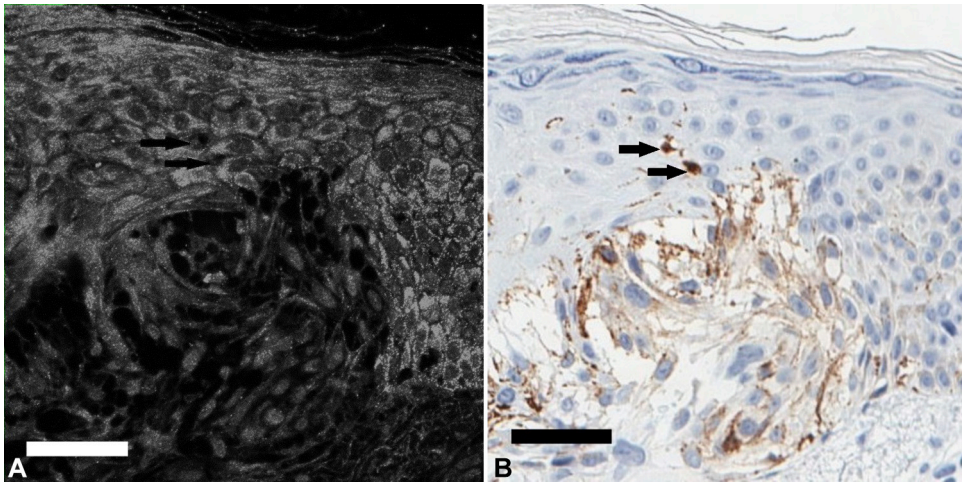

Fig 1. Spitz nevus, 3-mm diameter skin tumor with clear borders (56-year-old patient; nonpigmented skin color nevus on inner aspect of wrist; skin phototype II). **A**, Ex vivo reflectance confocal microscopy. **B**, Immunohistochemistry HMB 45 test. HMB 45^{+} intraepidermal melanocytes (*arrows*).

atypical immature melanocytes may have hyporeflective cytoplasm and a contrasting nucleus. This is an important finding because, generally, RCM melanoma diagnostics are based on hyperreflective cells and hyporeflective cells are ignored under usual diagnostics procedure. Hyporeflective pagetoid cells on in vivo RCM imaging were already reported by Losi et al,[5] but were not proved experimentally.[5]

To our knowledge, this is the first publication that correlates in vivo and ex vivo RCM with histopathological findings. It was found that neoplastic melanocytes might be hyporeflective in contrast to the surrounding keratinocytes, and this knowledge might improve in vivo RCM diagnostic analysis. We suggest that more studies on the combination of RCM and immunohistopathology of cutaneous melanocytic lesions would provide a better understanding of in vivo RCM imaging, its interpretation, and diagnostic accuracy of this promising technology.

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Indoor tanning among New Jersey high school students before and after the enactment of youth access restrictions

To the Editor: Individuals who indoor tan are at increased risk for melanoma, particularly if they start at a young age.[1] A total of 25 US states have age restrictions for the use of indoor tanning devices, and in December 2015 the US Food and Drug Administration issued a proposed rule to prohibit their use among those younger than 18 years.[2] Little is known about the impact of such laws on youth indoor tanning. We examined indoor tanning rates among New Jersey youth before and after a ban on indoor tanning for those younger than 17 years was enacted on October 1, 2013.

We used data from the 2012 and 2014 New Jersey Youth Tobacco Surveys, each of which used a cross-sectional design to survey a representative, statewide sample of public high school students (2012: N = 1850 students from 27 schools, 60.3% overall participation rate; 2014: N = 3909 students from 41 schools, 70.2% overall participation rate).[3] Parental consent and student assent procedures were used. The analyses for this study were deemed exempt from institutional review board review.

Students reported their sex, age, race/ethnicity, and the number of occasions they indoor tanned in the past year. The 2014 New Jersey Youth Tobacco Surveys was fielded from October 7 to December 15, 2014. Thus, students reported on their indoor tanning for the period when the ban was in effect for those younger than 17 years. The data were analyzed using SUDAAN software (RTI International, Research Triangle Park, NC) to take into account the complex sample survey design.

The samples from the 2012 and 2014 surveys did not differ significantly with regard to sex, age, or