Letter to the Editor

Photodynamic diagnosis of metastatic lymph nodes using 5-aminolevulinic acid in mouse squamous cell carcinoma

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To the Editor,

Squamous cell carcinoma (SCCs) is one of the most frequent types of non-melanoma skin cancer. In certain cases, SCCs spread to the regional lymph nodes (LNs), which results in poor prognosis. It is essential to accurately evaluate LN metastasis in order to select the appropriate therapeutic strategy and predict the outcomes of the patients with SCCs. In addition to using sentinel lymph node biopsy (SLNB), which is a potentially promising procedure for assessing LN metastasis in patients with SCCs[1], achieving accurate and rapid intraoperative diagnosis is vital to ensure the use of a less invasive surgery.

The photosensitizer 5-aminolevulinic acid (5-ALA) has recently been clinically used for detecting the primary lesion in certain types of non-melanoma skin cancers—this process is termed as photodynamic diagnosis. The exogenous administration of 5-ALA causes selective accumulation of the heme precursor protoporphyrin IX (PpIX) in cancer cells.[2] PpIX is a fluorescent substance that emits a strong red fluorescence at approximately 635 nm on blue light excitation.[3, 4]

In the present study, we aimed to evaluate the feasibility of using 5-ALA-induced PpIX fluorescence as a rapid intraoperative diagnostic tool for LN metastasis in mouse SCCs.

A mouse model of SCCs with LN metastasis was established, as previously described by Matsumoto *et al.* [5] with minor modifications. In brief, NR-S1M cells (6×10^6 cells) were intracutaneously injected into the back area—adjacent to the tail—of 6–8-week-old C3H/He mice (SHIMIZU Laboratory Supplies, Kyoto, Japan). Three to 4 weeks after tumor implantation, the mice were administered 5-ALA hydrochloride (Wako Pure Chemical Industries, Kyoto, Japan) at a dose of 250 mg/kg body weight through a tail vein injection.[6] Six to 9 hours after the injection, the inguinal, axillary, and para-aortic LNs were excised and examined using fluorescence stereomicroscopy, as described previously.[6, 7] Briefly, light emitting from a mercury lamp was filtered through a 405 ± 10 nm band-pass filter and used for excitation. The fluorescent emission at a wavelength longer than 430 nm was transmitted through a long-pass filter and detected by charge-coupled device camera. We performed intensity analysis by using Image-J software (National Institutes of Health, Bethesda, MD), as described previously.[7] The absolute mean R value and the signal intensity ratio of red to the sum of red, green, and blue ($R/[R+G+B]$) were used as indices

corresponding to the red fluorescence of PpIX. All procedures were performed in accordance with the guidelines of the Animal Care and Use Committees of Kyoto Prefectural University of Medicine.

In total, 33 LNs obtained from 7 mice were examined, 12 of which were metastatic LNs. Strong accumulation of PpIX fluorescence was detected within the metastatic lesions in metastatic LNs (Figure 1A). Dimly weak to moderate fluorescence in the entire LN was detected in most of the non-metastatic inguinal or axillary LNs (Figure 1B). However, para-aortic LNs without metastasis showed no fluorescence or imperceptible fluorescence (Figure 1C). Compared to that in non-metastatic LNs, the R value (Figure 2A) and R/(R+G+B) ratio (Figure 2B) indicated significant increments in metastatic LNs (R value: metastatic LNs, 69.92 ± 38.29 vs. non-metastatic LNs, 23.73 ± 19.07 , $P < 0.001$; R/(R+G+B) ratio: metastatic LNs, 0.782 ± 0.153 vs. non-metastatic LNs, 0.501 ± 0.154 , $P < 0.001$)

Thus, we observed that the evaluation of 5-ALA-induced PpIX fluorescence can be a useful method for detecting metastatic LNs in a mouse SCC model. To our knowledge, this is the first report that applied photodynamic diagnosis using 5-ALA for the detection of LN metastasis in SCCs. Histological analysis is commonly performed for detecting LN metastasis through rapid intraoperative diagnosis. However, several studies have reported that rapid intraoperative pathological diagnosis is associated with a significant risk of overlooking metastasis, as only a few hematoxylin and eosin-stained slides from whole LNs are examined during routine histological examinations.[8, 9]

In addition to SCCs, photodynamic diagnosis or therapy of the primary lesions is widely used for non-melanoma skin cancers as well, including extramammary Paget disease, wherein LN metastasis would result in a poor prognosis. As fluorescence imaging methods are simple, rapid, and easily applicable, we believe that the 5-ALA-induced PpIX fluorescence method for rapid intraoperative diagnosis can be a novel and promising tool for patients with various types of non-melanoma skin cancers.

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Figure Legends

Figure 1. Representative images of metastatic lymph nodes (A) and non-metastatic inguinal (B) and para-aortic (C) lymph nodes. White-light and fluorescence images (left panels, white images; middle panels, fluorescence images, scale bar: 1mm) and hematoxylin and eosin-stained sections (right panels, original magnification: $\times 12.5$) of each lymph node are shown. The yellow arrows indicate metastatic foci. Red fluorescence, representative of metastatic foci, is observed in the metastatic lymph node (A). Microscopic fluorescence images: excitation, 395–415 nm; emission, >430 nm.

Figure 2. The absolute mean R value (A) and the signal intensity ratio of red to the sum of red, green, and blue ($R/[R+G+B]$) (B) between metastatic lesions of metastatic lymph nodes and non-metastatic lymph nodes. (*, $P < 0.001$)

Figure 1

A

B

Figure 2