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Detecting tumour-positive resection margins after oral cancer surgery by spraying a fluorescent tracer activated by gamma-glutamyltranspeptidase

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ABSTRACT

Objectives: Tumour-positive resection margins are a major problem during oral cancer surgery. gGlu-HMRG is a tracer that becomes fluorescent upon activation by gamma-glutamyltranspeptidase (GGT). This study aims to investigate the combination of gGlu-HMRG and a clinical fluorescence imaging system for the detection of tumour-positive resection margins.

Materials and methods: The preclinical Maestro and clinical Artemis imaging systems were compared in vitro and ex vivo with cultured human head and neck cancer cells (OSC19, GGT-positive; and FaDu, GGT negative) and tumour-bearing nude mice. Subsequently, frozen sections of normal and oral cancer tissues were ex vivo sprayed with gGlu-HMRG to determine the sensitivity and specificity. Finally, resection margins of patients with suspected oral cancer were ex vivo sprayed with gGlu-HMRG to detect tumour-positive resection margins.

Results: Both systems could be used to detect gGlu-HMRG activation in vitro and ex vivo in GGT positive cancer cells. Sensitivity and specificity of gGlu-HMRG and the Artemis on frozen tissue samples was 80% and 87%, respectively. Seven patients undergoing surgery for suspected oral cancer were included. In three patients fluorescence was observed at the resection margin. Those margins were either tumour-positive or within 1 mm of tumour. The margins of the other patients were clear (≥8 mm).

Conclusion: This study demonstrates the feasibility to detect tumour-positive resection margins with gGlu-HMRG and a clinical fluorescence imaging system. Applying this technique would enable intraoperative screening of the entire resection margin and allow direct re-resection in case of tumour-positivity.

Introduction

Surgery remains the treatment of choice for the curative therapy of squamous cell carcinoma (SCC), including oral cancer. A resection requires wide margins to ensure no residual tumour tissue is left behind after surgery. However, such resections often cause functional loss. In the case of oral cancer, inadequate resection margins (i.e. close and positive margins) are reported in up to 85% of these patients [1,2]. These patients more often develop local recurrences and regional neck metastases, resulting in decreased survival rates [3]. Patients with oral cancer often have leucoplakia, which makes intraoperative discrimination between benign and malignant tissue challenging. Intraoperative frozen section analysis is sometimes used in oral cancer surgery to assess whether the resection margin is free of tumour.

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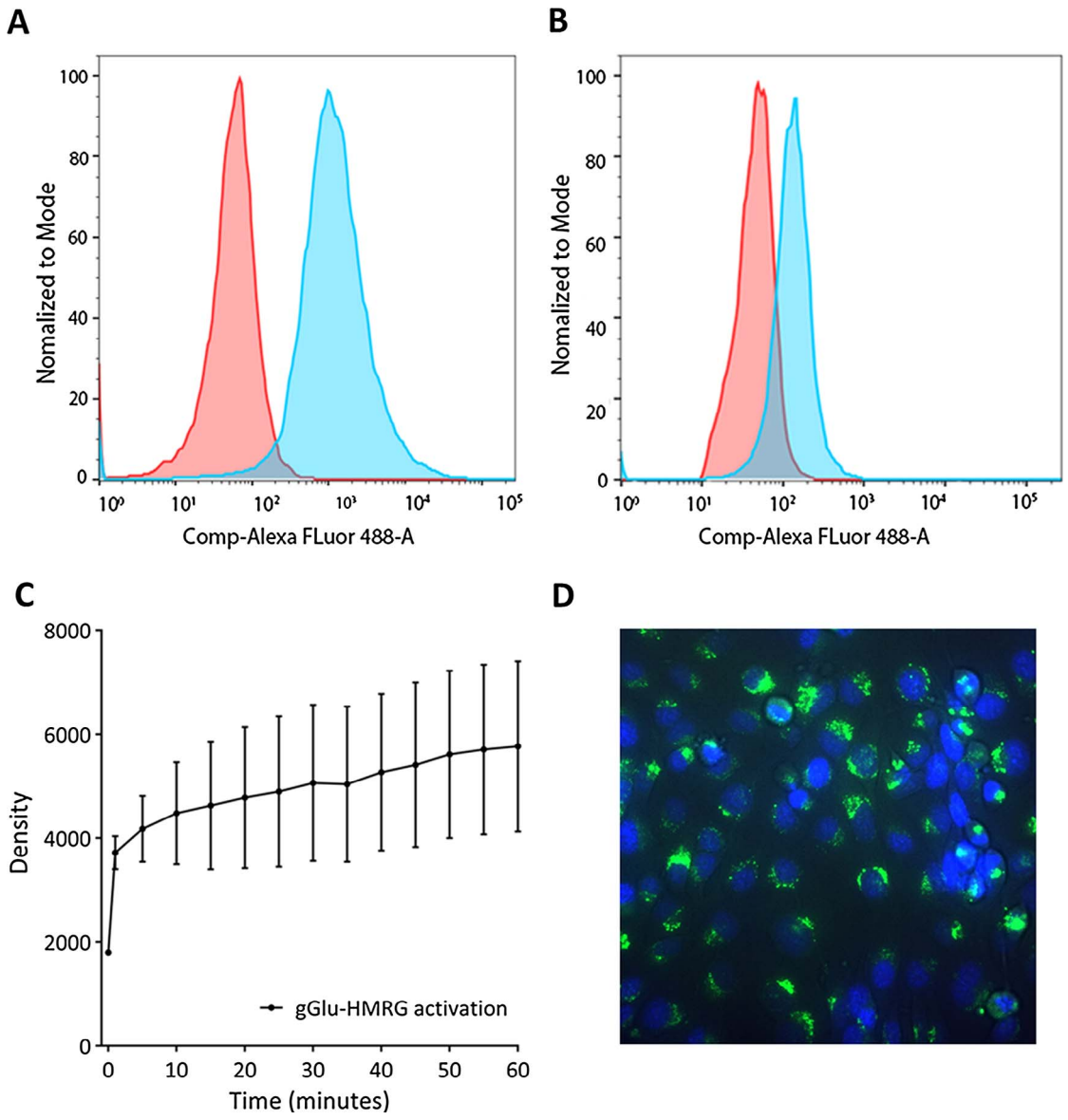

Fig. 1. Flow cytometry of the GGT-positive cell line OSC19 (A) and the negative cell line FaDu (B). The majority of the activation of gGlu-HMRG on OSC19 cells occurs within the first ten minutes (C). HMRG is internalized after activation (D; 40 times enlarged).

However, this method has certain drawbacks. Sampling errors can result in incorrect diagnosis. A pathologist is required for histopathological examination where the subsequent processing of the biopsy would then delay the surgical procedure [4,5]. Ideally, the entire resection surface should be evaluated during the operation, but this is not possible with current strategies.

Recently, several clinical studies with tumour-targeted fluorescent tracers demonstrated the feasibility to visualize tumours, and more importantly their margins, during surgery [6–8]. Cetuximab was conjugated to the near-infrared fluorescent dye IRDye800CW. The combination enabled demarcation of tumours with millimetre-resolution in oral cancer patients [9]. These results are promising, but these tracers have several disadvantages. First, intravenous administration may lead to adverse reactions. Cetuximab, for example, can cause severe infusion reactions and other harmful side effects [10]. Second, antibody-based tracers have long plasma half-lives, and intravenous administration requires relatively high doses to have sufficient tracers reach the tumour. Unbound tracers in the systemic circulation result in non-specific background fluorescence. This reduces the signal-to-background ratio (SBR) and potentially hampers visualization of specific fluorescence. Third, administration of labelled antibodies to patients needs to be applied several days before surgery, which requires an additional visit or earlier admission to the hospital. This requires additional planning and is inconvenient for patients. Fourth, clinical translation of novel fluorescent tracers is costly, time-consuming and requires specific expertise.[11]

All these issues can be solved by topically applying activatable fluorescent tracers instead. This strategy requires a much lower dose and does not suffer from nonspecific fluorescence from unbound tracers. Moreover, spraying activatable tracers on only resected tissue only would not require costly translational research. Furthermore, such an approach would completely diminishes the risk of adverse events.

Recently, γ-glutamyl hydroxymethyl rhodamine green (gGlu-HMRG) was developed for the detection and diagnosis of preclinical cancer [12]. HMRG remains quenched until cleaved in the presence of the enzyme gamma-glutamyltranspeptidase (GGT). After activation HMRG emits fluorescence with a peak at 525 nm. Application of gGlu-HMRG on resected material was investigated to identify tumour-positive resection margins in breast cancer patients [13]. Mizushima et al. [14] showed that fluorescence imaging with gGlu-HMRG could be