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In vivo imaging of lymph node metastasis with telomerase-specific replication-selective adenovirus

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Currently available methods for detection of tumors *in vivo* such as computed tomography and magnetic resonance imaging are not specific for tumors. Here we describe a new approach for visualizing tumors whose fluorescence can be detected using telomerase-specific replication-competent adenovirus expressing green fluorescent protein (GFP) (OBP-401). OBP-401 contains the replication cassette, in which the human telomerase reverse transcriptase (hTERT) promoter drives expression of *E1* genes, and the *GFP* gene for monitoring viral replication. When OBP-401 was intratumorally injected into HT29 tumors orthotopically implanted into the rectum in BALB/c *nu/nu* mice, para-aortic lymph node metastasis could be visualized at laparotomy under a three-chip color cooled charged-coupled device camera. Our results indicate that OBP-401 causes viral spread into the regional lymphatic area and selectively replicates in neoplastic lesions, resulting in GFP expression in metastatic lymph nodes. This technology is adaptable to detect lymph node metastasis *in vivo* as a preclinical model of surgical navigation.

Medical imaging techniques have become an essential aspect of cancer diagnosis, detection, and treatment monitoring. Advances and improvements in the major imaging modalities such as computed tomography, magnetic resonance imaging and ultrasound techniques have increased the sensitivity of visualizing tumors and their metastases in the body[1,2]. A limiting factor of these techniques is, however, the inability to specifically identify malignant tissues. Positron emission tomography with the glucose analog ^{18}F-2-deoxy-D-glucose is the first molecular imaging technique that has been widely applied for cancer imaging in clinical settings[3]. Although ^{18}F-2-deoxy-D-glucose–positron emission tomography has high detection sensitivity, it has some limitations such as the difficulty in distinguishing between proliferating tumor cells and inflammation and the inability in using it for real-time detection of tumor tissues. A relatively inexpensive, robust and straightforward way of defining the location and area of tumors *in vivo* would greatly aid the treatment of human cancer, especially for surgical procedures. In particular, if tumors too small for direct visual detection and therefore not detectable by direct inspection could be imaged *in situ*, surgeons could excise such tumors precisely with appropriate surgical margins.

Sentinel lymph node (SLN) mapping is a minimally invasive procedure and widely used in the management of patients with cutaneous melanoma or breast cancer without clinical evidence of nodal metastases[4,5]. The technique assumes that early lymphatic metastases, if present, are always found first within the SLN, the first tumor-draining lymph node. A SLN free of tumor cells would therefore predict the absence of metastatic disease in the rest of the tumor-draining lymph node basin, which indicates that intensive lymphadenectomy is unlikely to benefit those patients. Several studies have validated this assumption; the sensitivity of intraoperative frozen-section analysis for detection of nodal metastases, however, is relatively low, and high false-negative rates have been reported[6–9]. In addition, thicker primary and larger SLN tumor size has been shown to be predictive of non-SLN metastasis, presumably because of the altered lymphatic drainage routes. These findings raise doubt about the applicability of this technique in widespread surgical practice; therefore, several different approaches have been taken to directly label tumor cells to visualize and track them *in vivo*.

The GFP, which was originally identified from the jellyfish *Aequorea victoria*, is an attractive molecular marker for imaging in live tissues because of the relatively noninvasive nature of fluorescence[10–15]. We previously demonstrated a real-time fluorescence optical imaging of pleural dissemination of human non-small-cell lung cancer cells in an orthotopic mouse model using tumor-specific replication-competent adenovirus (OBP-301, Telomelysin)[16,17] in combination with replication-deficient adenovirus expressing *GFP* (Ad-*GFP*)[18]. In the present study we additionally modified OBP-301 to contain the *GFP* gene driven by the cytomegalovirus (CMV) promoter for monitoring viral replication. The resultant adenovirus, termed OBP-401, efficiently labeled tumor cells with green fluorescence *in vitro* and *in vivo*. The results showed that injection of OBP-401 into primary tumors allows its lymphatic spread, which in turn induces viral replication in

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