Real-time diagnostic imaging of tumors and metastases by use of a replication-competent herpes vector to facilitate minimally invasive oncological surgery

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To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.05-5316fje; doi: 10.1096/fj.05-5316fje

SPECIFIC AIMS

1. To establish whether a strain of HSV-1, NV1066, could delineate tumor tissue from normal tissue

We sought to determine whether tumor-specific targeting of NV1066 can help delineate tumor tissue from normal tissue in a wide range of cancers.

2. To determine whether NV1066-induced GFP expression can detect small foci of tumors and metastases in in vivo models

The diagnostic potential of HSV guided fluorescence stems from its ability to specifically infect and replicate within tumor cells. This results in cancer-specific expression of GFP that may identify even small foci of malignant tissue.

3. To determine whether cancer cell-specific viral production of GFP can be useful in various clinical scenarios to promote use of minimally invasive oncological surgery

We experimented to see whether cancer cell-specific viral production of GFP can be used for real-time intraoperative imaging and enhanced detection of early cancers and metastases. Because HSV is replication-competent, we tested whether a small initial dose of virus can spread to other cancer cells and express GFP.

PRINCIPAL FINDINGS

1. NV1066 infects and expresses fluorescence in a wide range of cancer cells

In vitro, NV1066 infected and expressed GFP in more than 100 cancer cell lines from 16 different primary organs. The ability to infect and express fluorescence is present regardless of the phenotypic or pathological variability of the cancer cells. NV1066 expressed GFP even at low doses starting as early as 2–4 h after administration. The GFP is expressed intracellularly and can be detected by fluorescent microscopy and by flow cytometry. Molecular confirmation was obtained using immunohistochemistry to show that the intracellular green fluorescence is due to viral infection. The intensity of GFP expression is directly related to the cellular proliferation rate with the highest degree of fluorescence found within rapidly dividing cells. As NV1066 replicates within cancer cells, initial low doses of virus still results in adequate green fluorescence. NV1066 replication within cancer cells is demonstrated by the 5- to 3000-fold increase in viral titers as measured by viral plaque assay.

2. Fluorescence-guided minimally invasive detection of abdominal tumor and metastases

NV1066 infected all cancer tissue even when a single low dose of virus (2.5 or 5 × 106 plaque forming units, PFU) was injected into the abdominal cavity. Under fluorescent laparoscopy, tiny cancerous nodules are clearly identified and delineated from autofluorescence at multiple sites in the mesentery on intestinal loops, in the pararectal space, and on the peritoneum. Selective biopsy of the fluorescent lesions facilitated accurate diagnosis of small tumor deposits that were missed on routine bright-field laparoscopic examination. Endoluminal evaluation by upper gastrointestinal endoscopy also detected early tumors. The efficacy of NV1066 is maintained regardless of the method of administration, whether by intratumoral, intracavitary, or systemic tail vein injection.

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3. Fluorescent thoracoscopy enhances tumor detection

A single dose of NV1066 instilled into the thoracic cavity is able to spread, infect, and express green fluorescence in tumor tissue while the normal lung and heart tissue is spared (Fig. 1).

4. NV1066-directed fluorescence reveals the location of metastases

Injection of NV1066 into the primary tumor results in dissemination of virus to metastatic sites to allow identification of metastatic disease with endoscopic examination. A single dose of NV1066 injected into the primary breast tumor resulted in the infection and fluorescence of multiple metastatic lung tumors. A single-dose, systemic injection of NV1066 for esophageal cancer revealed selective infection and fluorescence of liver metastases. Splenic metastases from pleural mesothelioma were identified after injection of NV1066 into the chest cavity. Similarly, systemic injection of NV1066 clearly delineated adrenal and renal metastases in a primary lung cancer model. Thus, administration of diagnostic virus does not need to be at each site of metastatic disease for use in identification of disseminated cancer.

5. Minimally invasive detection of lymph node metastases

Administration of NV1066 into the primary tumor resulted in spread by the lymphatics, revealing metastases in both the regional stations and in distant nodes. NV1066-guided fluorescence delineated cervical, axillary, celiac, and para-aortic lymph nodes diseases after viral administration into the primary cancer that is distant from the identified lymph nodes.

6. GFP-fluorescence enables detection of micrometastatic tumors

Green fluorescence identified tumor deposits, localizing even microscopic collections of tumor cells (<1 mm) not apparent under bright-field endoscopy. Non-tumor-bearing thoracic and abdominal organs did not fluoresce in vivo when examined through the GFP filter and were easily distinguished from infected tumor deposits. The presence of tumor cells was confirmed by histology and immunohistochemistry. Topographic maps of GFP expression were generated from the digital images obtained with the fluorescent microscopic or endoscopic systems (Fig. 2). This technology enables real-time identification and quantification of in vivo fluorescent intensity.

7. A single dose of NV1066 is able to spread across cavities and express GFP in tumor and metastases

To confirm that NV1066-guided fluorescence detection of tumors and metastases is not isolated to the body cavity of viral injection, we created advanced tumor models with widespread dissemination of metastases. When a single dose of NV1066 (5 × 10⁶ PFU) is injected into either the chest or abdominal cavity, virus spreads to the other cavity and infects metastases. In mice with pleural cancer, a single intrapleural injection of NV1066 identified tumor deposits in the para-aortic lymph nodes, adrenal, and splenic metastases.

8. NV1066 specificity targeting tumor tissue and metastases is confirmed by histology, immunohistochemistry and real-time PCR for HSV-1

After pleural administration of NV1066, strong GFP expression was noted in the pathological sections of
tumor nodules (Fig. 1C). All sections that expressed GFP were found to have tumor cell infiltrates corresponding to areas of expression (Fig. 1C). H & E staining confirmed that GFP expression localized to foci of cancer (Fig. 1D). Staining for polyclonal HSV-1 antibody also corresponded to areas of GFP expression by histology (Fig. 1D). No viral staining was evident in tissues that did not express GFP. Solid organs, serum, and tumor nodules were analyzed for the presence of viral gene by PCR. There was no viral gene presence detected in the normal organs and serum 3 days after viral inoculation. In tumor tissue, viral gene presence amplified from 7- to 47-fold confirming viral infection and replication. The level of viral gene amplification correlates with the length of time after viral injection.

9. NV1066 specifically targets spontaneous tumors and spares normal tissue

To confirm that NV1066 infection is not isolated to immunodeficient animal models, murine SCC VII syn-

geneic subcutaneous tumors were grown in C3H/HeJ immunocompetent mice. Injection of NV1066 into the primary tumor selectively infected only cancerous tissue, sparing normal tissue. NV1066 tumor specificity was further demonstrated in two spontaneous tumor models in immunocompetent animals; SENCAR mice and hamsters. In both animal models, carcinogen application (DMBA, dimethyl benzanthracene) resulted in spontaneous cancer development 8–16 wk after exposure. These tumor models were selected because their spontaneous tumor development most closely mimics the physiological progression of cancer. NV1066 tumor specificity is confirmed with fluorescence imaging and immunohistochemistry to prove that the tumor targeting specificity of NV1066 is not species dependent and is capable of functioning in hosts with intact immune systems.

CONCLUSIONS AND SIGNIFICANCE

This study expands the use of HSV-guided fluorescence from solid tumors and into its use within body cavities and demonstrates that the administered virus can travel along natural paths of spread of cancer, including intracavity, vascular, and lymphatic, to reach and identify metastatic disease. Primary microscopic tumor deposits are evident as are nodal and distant metastases. As HSV is replication-competent, a small initial dose of virus spreads to other cells eventually turning the entire tumor green. We noted that NV1066 spreads and expresses fluorescence on the surface of tumors in body cavities at a faster rate. This ability is important when HSV is used solely for diagnostic staging purpose, where a low dose of virus can identify cancerous tissue.

Figure 2. Endoscopic identification of micrometastatic tumor deposits. NV1066 specificity to microscopic tumor nodules enables clear in vivo identification of microscopic tumor deposits down to even less than a millimeter in size. Tumor deposits not clearly identified on white light thoracoscopy (A, B) could be identified by GFP expression with fluorescent endoscopy. Topography maps of GFP expression were digitally created to quantify fluorescent intensity relative to background (C). GFP, green fluorescent protein.

Figure 3. Diagnostic imaging possibilities using herpes simplex virus.