LABORATORY INVESTIGATION

Near-Infrared Fluorescence Imaging of Matrix Metalloproteinase 2 Activity as a Biomarker of Vascular Remodeling in Hemodialysis Access

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ABSTRACT

Purpose: To establish the capability of near-infrared fluorescence (NIRF) imaging for the detection of matrix metalloproteinase 2 (MMP-2) activity as a biomarker of vascular remodeling (VR) in arteriovenous fistulae (AVFs) in vivo.

Materials and Methods: AVFs were created in the right groins of Wistar rats (n = 10), and sham procedures were performed in the contralateral groins. Fistulography via a left common carotid artery approach confirmed stenosis (> 50%) in a subset of animals (n = 5) 4 weeks after AVF creation. After administration of MMP-2–activated NIRF probe, near-infrared imaging was performed in vivo and ex vivo of both the AVF and the sham-treated vessels to measure radiant efficiency of MMP-2–activated NIRF signal over background. Histologic analyses of AVF and sham-treated vessels were performed to measure VR defined as inward growth of the vessel caused by intimal thickening.

Results: AVFs demonstrated a significantly higher percentage increase in radiant efficiency over background compared with sham vessels (45.5 ± 56% vs 16.1 ± 17.8%; $P = .008$). VR in AVFs was associated with increased thickness of neointima staining positively for MMP-2 (161.8 ± 45.5 μm vs 73.2 ± 36.7 μm; $P = .01$). A significant correlation was observed between MMP-2 activity as measured by relative increase in radiant efficiency for AVFs and thickness of neointima staining positively for MMP-2 ($P = .039$).

Conclusions: NIRF imaging can detect increased MMP activity in remodeled AVFs compared with contralateral sham vessels. MMP-2–activated NIRF signal correlates with the severity of intimal thickening. These findings suggest NIRF imaging of MMP-2 may be used as a biomarker of the vascular remodeling underlying stenosis.

ABBREVIATIONS

AVF = arteriovenous fistula, AVG = arteriovenous graft, MMP-2 = matrix metalloproteinase 2, NIRF = near-infrared fluorescence, VR = vascular remodeling

Approximately 82% of end-stage renal disease patients receive hemodialysis through an arteriovenous graft (AVG) or arteriovenous fistula (AVF) (1). However, low patency rates are a frequent cause of morbidity, with primary patency rates for AVFs and AVGs at 1 year between 60% and 70% and thrombosis as the most common cause of access failure and abandonment (2–6). Stenosis results from negative vascular remodeling (VR) characterized by inward growth

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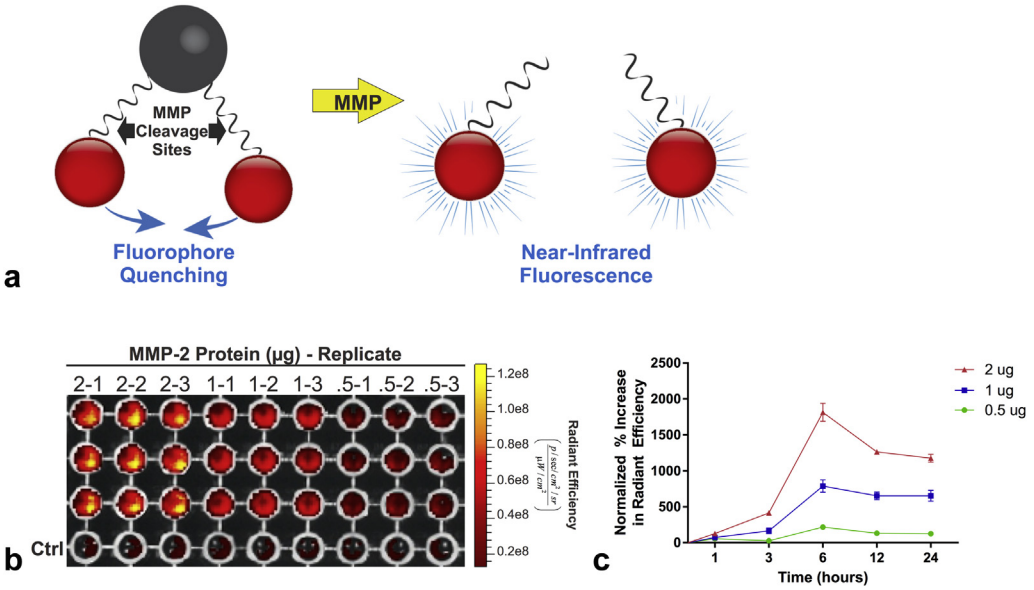

Figure 1. In vitro characterization of the MMP-2–activated probe. (**a**) Illustration of proteolytic activation of MMPSense 750 by MMP-2 resulting in relief of autoquenching and resultant near-infrared fluorescence. (**b**) Representative near-infrared fluorescence image of MMPSense activation by serial amounts of recombinant MMP-2 protein in vitro. Data are presented as three experimental replicates. (**c**) Associated scatter plot demonstrating normalized percentage increase in radiant efficiency over control samples after serial incubations of MMPSense with the indicated amount of recombinant MMP-2 protein.

of the vessel by neointimal hyperplasia (NH). Progressive NH culminates in luminal narrowing, increased resistance, decreased blood flow, and ultimately thrombosis (7). Among the molecular mediators of negative VR, matrix metalloproteinase 2 (MMP-2) is of particular importance. MMP-2 promotes the differentiation of adventitial fibroblasts to myofibroblasts and plays an essential role in the degradation of extracellular matrix enabling cellular migration into the intima (8–11). MMP-2 is significantly increased in stenotic regions of AVGs and AVFs (7,8,12,13).

Current methods of evaluating malfunctioning HD access detect the late sequelae of VR and its related impairment of adequate HD. Although a variety of imaging modalities have been applied to evaluate malfunctioning HD access, including fistulography (the criterion standard), ultrasound, and magnetic resonance imaging, they are limited to measuring late functional, morphologic, and/or structural changes of VR. In contrast, near-infrared fluorescence (NIRF) optical imaging has the potential to image the molecular alterations that initiate NH and precede the anatomic changes of VR. NIRF imaging of MMP-2 activity has been demonstrated in models of vascular pathology and malignancy (14–16). This technique represents a unique resource to translate the knowledge of the molecular pathogenesis of negative VR in HD access into a minimally invasive, clinically applicable imaging paradigm for the detection of impending stenosis and to facilitate the development of new therapies. Therefore, it was hypothesized that NIRF imaging of MMP-2 activity in a nonhuman animal model of AVF could serve as an imaging biomarker for vascular remodeling in HD access.

MATERIALS AND METHODS

All experiments were performed in accordance with institutional guidelines and approval of the Institutional Animal Care and Use Committee.

In Vitro Characterization of MMP-2–Activated Near-Infrared Fluorescent Probe

In vitro characterization of the MMP–activated NIRF probe (MMPSense 750; Perkin Elmer, Waltham, Massachusetts) was performed with the use of activated recombinant MMP-2 (Sigma-Aldrich, St. Louis, Missouri). MMPSense 750 is a biomolecule consisting of a backbone of 8 amino acids, which can be cleaved by MMPs. Two near-infrared spectrum fluorophores (excitation wavelength 749 nm, emission wavelength 775 nm) are linked to this amino acid chain in close proximity to one another. In the uncleaved state, the proximity of the fluorophores results in partial quenching of the signal upon excitation. This quenching phenomenon is abrogated when the backbone undergoes proteolytic cleavage by an MMP (Fig 1a).

In vitro assessments of MMP-2 specific activation of MMPSense 750 were performed by incubating 500 nmol/L of the fluorophore with serial dilutions of activated MMP-2 (0.25, 0.5, 1, and 2 μg) in phosphate-buffered saline solution (PBS) incubated at 37°C in 96-well tissue culture plates. At 1, 3, 6, 12, and 24 hours of incubation, the samples were imaged with the use of an IVIS Spectrum Imaging System (Perkin Elmer, Waltham, Massachusetts) at excitation and detection wavelengths of 750 nm and 780 nm, respectively. Total radiant efficiency (RE) of each sample was calculated