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Molecular Sonography: Current and Future Applications

Arthur Fleischer and Sai Chennupati

Abstract

This communication provides an overview of the current and future applications of molecular sonography, emphasizing the principles of the technique. Molecular sonography is currently used for preclinical assessment of tumor detection and response in a variety of models. It has potential clinical applications in improved characterization of tumors based on their genomes. Clinical trials have been conducted for a variety of neoplastic, inflammatory and immunologic abnormalities.

Keywords: Labeled microbubbles, molecular characterization and detection, assessment of tumor response, ultrasonography

1. Introduction

Molecular imaging describes a relatively new imaging technique that depicts molecular processes rather than anatomic changes that occur in diseased tissue. It affords non-invasive imaging on a cellular level based on depiction of selected molecular receptors. The premise of molecular imaging is based on the fact that molecular events precede anatomic changes, and therefore, have the potential to detect neoplastic processes early as well as potentially determine response to therapy shortly after its administration. Molecular imaging can depict genomic phenotypes, and advantage that proves key to personalizing medical therapy.

There are a variety of modalities currently used to image diseased tissue. PET, or Positron Emission Tomography, utilizes radioactive substances known as tracers to detect disease. It is noninvasive and can identify areas of high metabolic activity that may be concerning for cancer. However, PET's anatomic clarity is imprecise compared to CT/MRI and it remains quite expensive. SPECT, Single Photon Emission Tomography, implements a similar mechanism as PET but is appreciably cheaper. Additionally, the radio tracers in SPECT have much longer half-lives, extending the imaging window. However, SPECT has even lower image resolution than PET. MRI, Magnetic Resonance Imaging, does not use radiation and provides a snapshot of the patient's anatomy with excellent resolution. However, it is notably time-consuming and expensive.

Molecular sonography holds many advantages over these imaging techniques. In addition to its low cost and high spatial resolution, molecular sonography is considered easy to use and repeatable when compared to most other diagnostic modalities. Currently, it is used for evaluation of tumor models. Reports of the use of molecular sonography in human subjects have recently been published [1].

This review will describe the current uses of molecular sonography and discuss future directions for research and development.

2. Targeted microbubbles

Microbubbles used for contrast enhanced and molecular sonography consist of an encapsulated gas covered by a lipid shell. They range from 2 to 5 microns in size, approximately one-third the size of an erythrocyte. As such, they remain intravascular and do not extravasate into the interstitium. They enhance the signal-to-noise ratio due to their oscillations in an insonated field as best depicted using harmonics.

A variety of targeting ligands, such as the vasogenic growth factor (VEGF) receptor, can be attached to the shell of a microbubble using the streptavidin-biotin spacer (**Figure 1**) [2]. Molecular sonography has been used to evaluate tumor angiogenesis, anti-immune encephalomyelitis, inflammatory bowel disease, transplant rejection and abnormal myocardial perfusion. The most frequently utilized application of molecular sonography involves a VEGF receptor antibody can be used as a ligand on a microbubble to detect tumor microvessels (**Figure 2**) [3]. This has shown to provide early detection of several cancers in murine models, notably pancreatic, prostate, and squamous cell carcinoma.

Labeled microbubbles may have an important role in monitoring tumor response to anti-angiogenic medications. In a preclinical proof of concept study using colon cancer models, 3D molecular sonography showed close correlation with treatment response to antiangiogenetic therapy [4].

Microbubble sonography can be used to assess the potential efficacy of specific anti-angiogenetic medications. In a pilot study of patients with refractory hepatocellular carcinomas, we found that a significant decrease in vascularity was seen in responsive vs. non-responsive tumors in the first 15 days after treatment was initiated (**Figure 3**) [5].

Similarly, molecular sonography has been studied as a means to evaluate acute ileitis in a swine model that targeted E- and P-selectin. The intensity of the sonographic signal correlated to the amount of immunofluorescence [6].

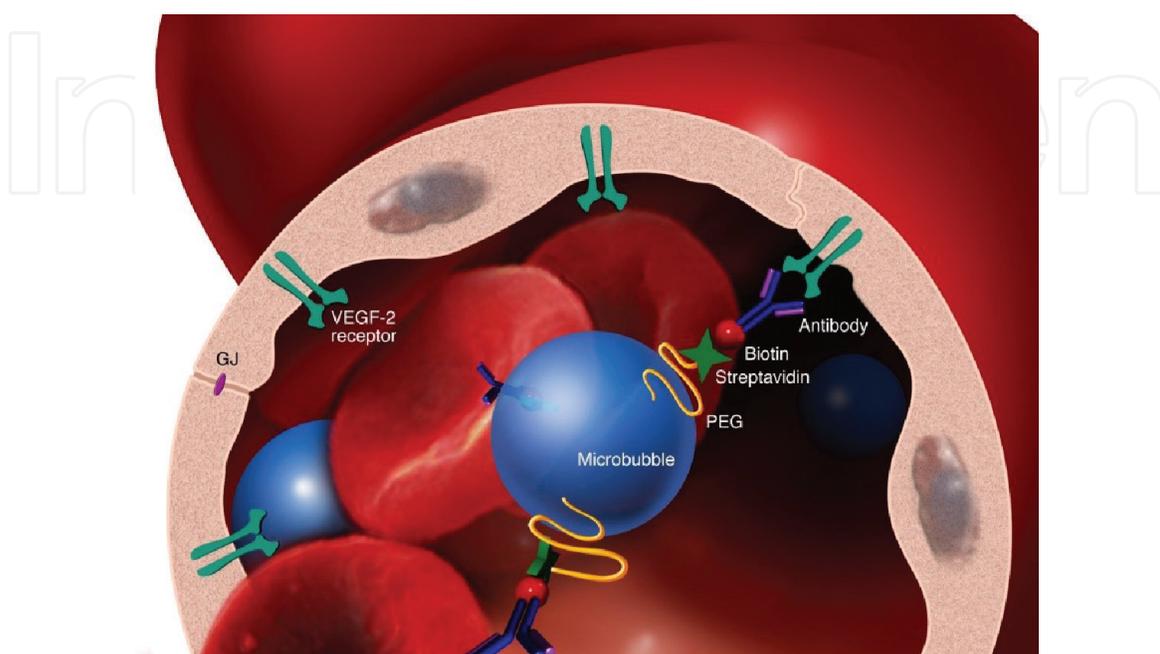


Figure 1. Drawing showing the streptavidin/biotin spacer for attachment of anti-VEGF receptor to microbubble.

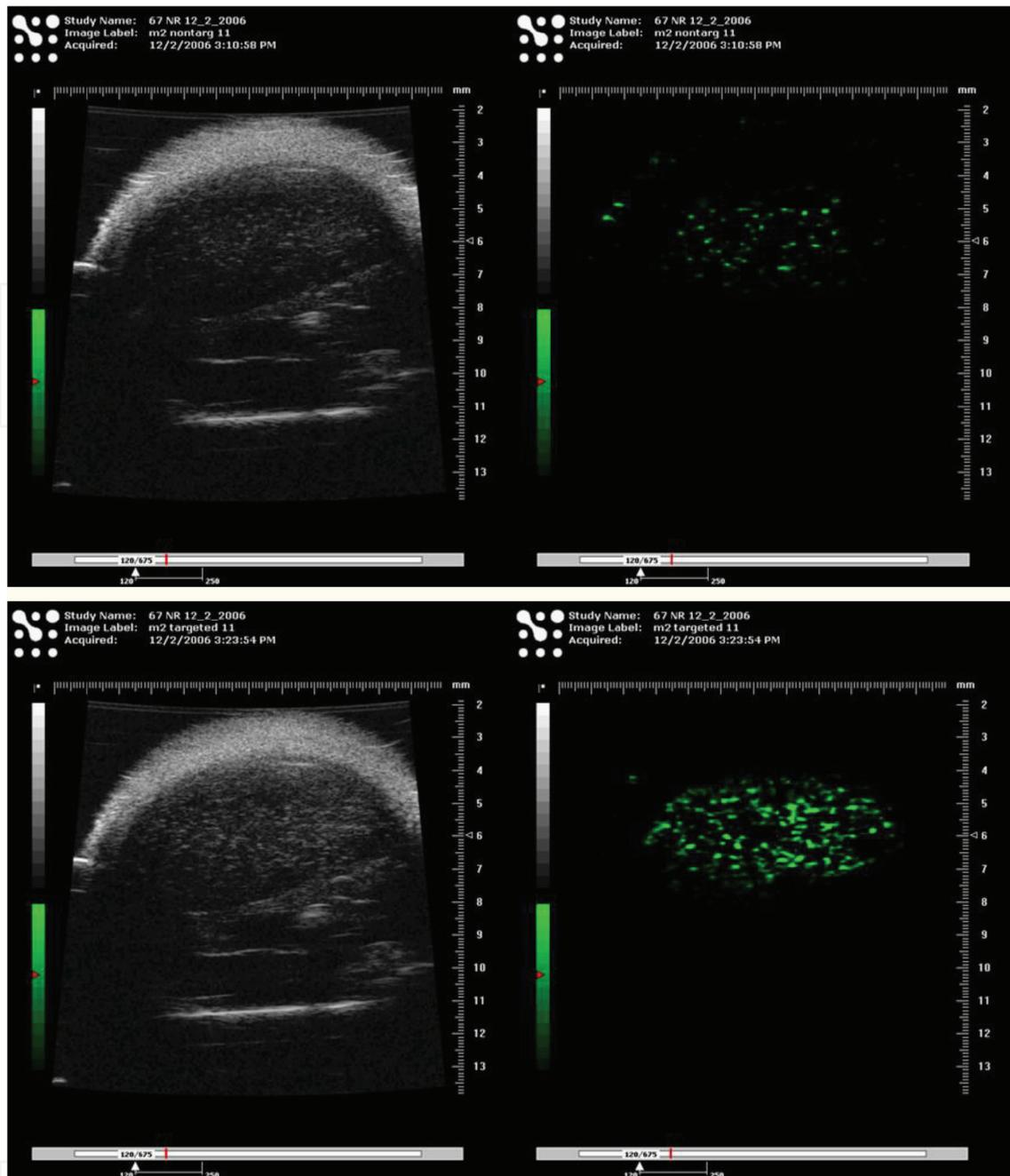


Figure 2. Molecular sonograms of implanted murine tumor with low (top) and high (bottom) VEGF receptors as confirmed with immunofluorescence [2].

3. Potential clinical applications

The main potential clinical application of labeled microbubbles includes early detection of tumors, monitoring tumor response, assessment of inflammation and/or ischemia, early detection of transplant infection and potential for targeted drug delivery.

One of the challenges to use these microbubbles is their fabrication for clinical use. Specifically, the immunogenicity of the streptavidin used for attaching the ligand to the bubble requires that it be internalized within the microbubble. This is only available from a few manufacturers on an investigational basis. We reported our early experience developing a labeled microbubble [7].

Only a few studies using labeled microbubbles in humans have been reported [1]. The group headed by Willman showed that breast and ovarian malignant

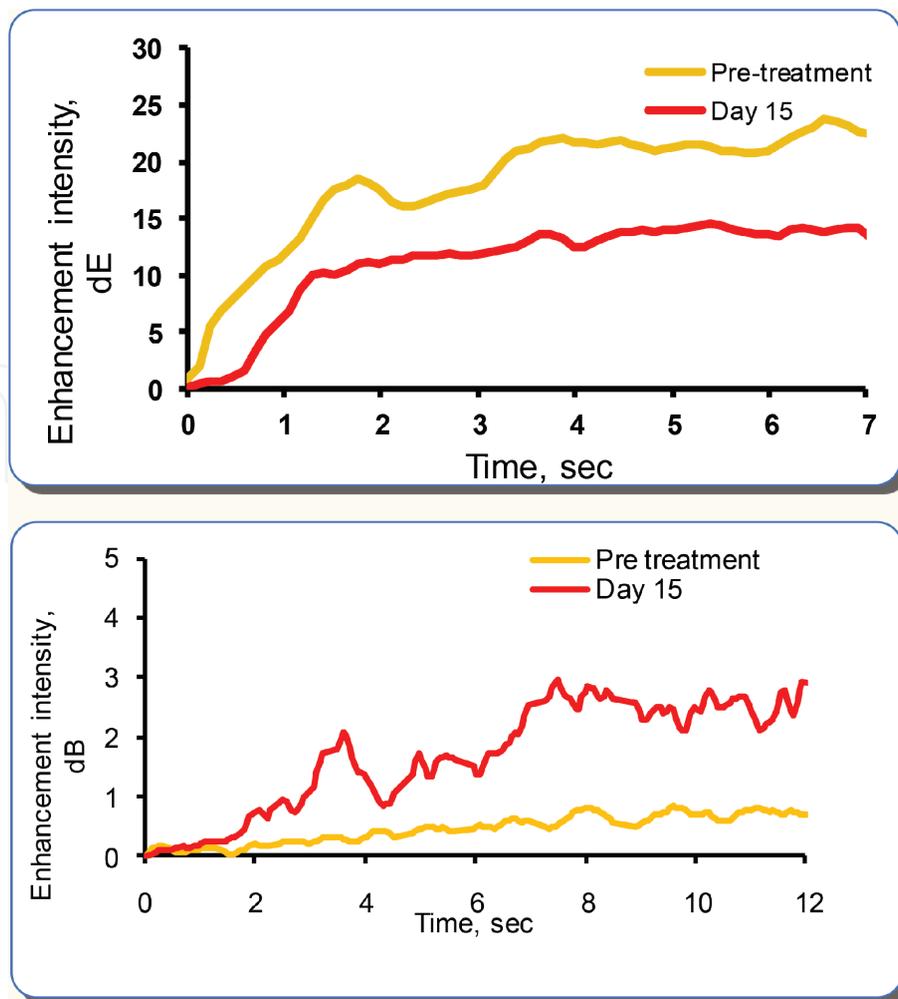


Figure 3. Time intensity curve in responsive (top) vs. non-responsive (bottom) hepatocellular cancer treated with anti-angiogenic medications.

tumors had greater signal than benign ones. Specifically, in a study of 24 patients with ovarian cancers and 21 with breast lesions, 93% of malignant breast lesions and 77% of malignant ovarian cancers demonstrated increased signal [1].

Labeled microbubbles have also been used in detecting prostate cancer and sentinel lymph nodes [7, 8]. Molecular lymphosonography was used to detect metastatic involvement in a swine melanoma model. Metastatic involvement of the sentinel lymph node can distinguish local vs. metastatic disease in women with breast cancer. It has also been used in a variety of cancers such as melanoma, esophageal, head and neck, stomach and thyroid cancer. Molecular sonography using nanobubble targeted prostate specific membrane antigen has been investigated in an orthotopic murine model [8]. Increased extravasation and retention were observed in prostate cancer models as well as exhibiting different time/intensity curves (enhancement kinetics) in cancerous tissue.

Angiogenesis, the generation of new blood vessels, has also been extensively investigated with molecular sonography due to its role in the development and metastatic spread of numerous cancers. It is crucial in the growth of tumors and subsequent metastasis. VEGFR2 is one of the most studied markers of angiogenesis and is over expressed on tumor-associated endothelial cells [9]. In one study of a transgenic mouse model, microbubbles targeting VEGFR2 showed an increased contrast-enhanced ultrasound signal from hyperplasia to ductal carcinoma in situ and breast cancer compared to normal breast tissue [10]. In another study of laying hens, a microbubble targeting $\alpha v \beta 3$ integrin sensitively detected ovarian cancer

at an early stage [11]. These are just two studies that support the clinical utility of angiogenesis targeted microbubbles in early tumor detection.

Applying the principles of molecular sonography to atherosclerosis can help identify at-risk plaques before acute events occur, potentially enabling prevention of evolution into irreversible damage. Normally, patients do not exhibit symptoms until an episode of severe stenosis or rupture occurs; thus, there is valuable utility in monitoring the growth of atherosclerotic plaques. JAM-A is an example of a marker that has been linked to early plaque formation and vulnerability [12]. In a study performed on mice that were fed an atherogenic diet, molecular ultrasound of JAM-A showed early stages of atherosclerosis and detected acute blood flow variations [13].

Acute cardiac ischemia is another pathology that would benefit from molecular ultrasound imaging. Current methods of detection such as EKG and cardiac MRIs suffer from various shortcomings such as accuracy, convenience, or safety. Sonography with labeled microbubbles could resolve a number of these shortcomings when used to identify post-ischemic myocardium. E- and P- selectin are two potential markers that were used in murine models to identify ischemic tissue shortly after undergoing an ischemic event [14, 15]. While cardiac molecular ultrasound imaging has shown promising results regarding safety and efficacy, it is technically difficult and highly user-dependent. Further testing needs to be performed to assess its feasibility when compared with current gold standards.

Microbubbles also can be applied in the assessment of inflammatory bowel disease (IBD). Two methods of assessing IBD are endoscopy and ultrasound. The former requires a procedure under anesthesia, while the latter is restricted by its relatively limited sensitivity. Molecular ultrasound holds clinical potential to serve as a complementary tool with high sensitivity in the imaging of IBD. One study utilized porcine tissue with terminal ileitis imaged with dual E- and P-selectin-targeted microbubbles. The study concluded that increased ultrasound signal correlated well with histologic grades of inflammation [16]. This study highlights selectin-targeted ultrasound's utility to serve as a high-sensitivity adjunctive tool for assessment of IBD in the clinical setting.

4. Conclusions

Molecular sonography has great potential as a means to detect early stages of a variety of cancers as well as a means to evaluate tumor response. It is anticipated that more widespread clinical use will occur in the next few years once these microbubbles become more readily available.

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Conflict of interest

The authors declare no conflicts of interest.

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