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Model system using controlled receptor expression for evaluating targeted ultrasound contrast agents

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Abstract

This report details a model system for evaluating targeted ultrasound (US) contrast agents using adenoviral (Ad) vectors to regulate target receptor expression. Receptor density *in vitro* was modulated in breast cancer cells by varying the multiplicity of infection (MOI) from 0 to 100. Target receptors were induced using a GFP reporter Ad vector for gene transfer and expression of the hemagglutinin (HA) tag. These reporter genes were under the control of the ubiquitous cytomegalovirus (CMV) promoter. Subsequently, receptor expression and anti-HA antibody (Ab) binding was examined with flow cytometry. Targeted US contrast agents, or microbubbles (MB), were created by conjugating either biotinylated anti-HA or isotype control Ab to the surface of biotin coated MBs via a streptavidin bridge. Targeted MBs were incubated with Ad infected 2LMP cells to evaluate *in vitro* MB binding. Experimental results found GFP expression to be directly correlated with Ad MOI ($r^2 = 0.96$). Increasing the Ad MOI produced a corresponding increase in binding and accumulation of anti-HA Ab on the cell surface ($p < 0.01$). However, no difference was found between Cy5-labeled anti-HA Ab exposed cell groups at an MOI of 0 ($p > 0.29$). Additionally, no difference was found between the isotype control Ab group ($p > 0.44$) indicating minimal nonspecific binding. No difference was found between cell groups incubated with isotype-targeted MBs ($p > 0.42$) regardless of receptor density. However, cells exposed to HA-targeted MBs showed increased levels of cell binding proportional to induced receptor expression levels ($p < 0.02$).

Keywords

Adenoviral vectors; Modulated receptors; Contrast enhanced ultrasound imaging; Microbubbles

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INTRODUCTION

Molecular imaging is a promising tool for the noninvasive visualization of molecular and cellular events inside the body. Ultrasound (US) has emerged recently as a promising molecular imaging strategy with the advent of microbubble (MB) contrast agents. These gas-filled MBs have an acoustic impedance mismatch compared to the surrounding blood leading to enhanced US backscatter signals during imaging (Leighton 1997). The surface of MBs can also be coated with ligands whose target receptors are overexpressed in a tissue of interest. Targeted ligands can be conjugated to the shell of the MB through either biotin-avidin chemistry or covalent linkage (Lindner et al. 2004). While unbound circulating MBs are cleared from the bloodstream within minutes, targeted agents bind to the specific endothelial markers providing contrast enhancement via localized accumulation (Klibanov et al. 2005). Recent studies have demonstrated the benefit of targeted MBs for US molecular imaging of tumor angiogenesis (Leong-Poi et al. 2002; Lindner 2004; Weller et al. 2002; Korpanty et al. 2007; Lyshchik et al. 2007; Willmann et al. 2008, 2010; Anderson et al. 2010; Knowles et al. 2011; Warram et al. 2011), inflammation (Christiansen et al. 2002; Klibanov et al. 2006; Korpanty et al. 2007; Behm et al. 2008; Lindner 2009; Kaufmann et al. 2009) and intravascular thrombi (Kaufmann et al. 2007; Kaufmann et al. 2009; Unger et al. 1998).

In cancer research, molecular imaging permits tumor localization in addition to detection of specific molecules associated with tumor growth and proliferation (Floyd et al. 2004). Targeting receptors involved in angiogenesis, such as vascular endothelial growth factor receptor 2 (VEGFR2) and $\alpha_v\beta_3$, have shown promise for improving contrast-enhanced US imaging of tumor vasculature (Willmann et al. 2008; Friedlander et al. 1995; Leong-Poi et al. 2002). For imaging inflammation, common receptors for targeted MBs include P-selectin, VCAM-1, and ICAM-1, which would allow for an assessment of atherosclerotic plaques, ischemia, and transplant rejection (Kaufmann et al. 2007). For contrast-enhanced US of cardiac tissue, molecular receptors such as P-selectin, VCAM-1, and glycoprotein IIb/IIIa are commonly explored in preclinical settings to improve diagnosis of deep venous thrombosis, intracardiac thrombi, and atherosclerotic lesions (Kaufmann et al. 2007, 2009).

Microbubble targeting strategies have thus been used to detect the expression of disease-specific receptors in tissue. However, the relation between receptor site density and microbubble attachment has in the past been difficult to assess. A closer look at binding dynamics through receptor density alteration would benefit the field of targeted contrast-enhanced US by removing bias of varying receptor expression within a cell line. Using a dual-reporter adenovirus previously described (Zinn et al. 2002), expression of an exogenous cellular receptor, hemmagglutinin (HA) and a fluorescent imaging reporter, green fluorescent protein (GFP), both under the control of a constitutive promoter (cytomegalovirus, CMV), can be modulated by altering the multiplicity of infection (MOI). The MOI represents the ratio of infectious particles to the cells available for infection. The HA tag is not found in normal tissue and serves as a specific marker of gene transfer. Thus, controlling the Ad vector MOI allows modulation of cell surface receptor and fluorescent protein expression.

Targeted US contrast agents have been the focus of research activity for more than a decade. Experimental studies on the effect of variable receptor density on MB binding have been inherently limited to choosing different cell lines given their associated (different) intrinsic receptor profiles. No model systems are currently available for controlling receptor expression and subsequently evaluating targeted US contrast agents. Therefore, the aim of this study was to develop and validate a model system for modulating receptor expression

using Ad vector techniques and assessing targeted MB binding through *in vitro* experimentation.

MATERIALS AND METHODS

Cell culture

2LMP (MDA-MB-231 lung metastatic pooled, kindly provided by Dr. Donald Buchsbaum, UAB) breast cancer cells were grown in Dulbecco's Modified Eagles medium without phenol red (Mediatech, Inc, Manassas, VA) with 10% FBS (HyClone, Loga, UT) and 1% L-Glutamine. Cells were trypsinized and collected at 80% confluency and counted with a hemocytometer for the *in vitro* assay. Cell viability was determined using trypan dye exclusion. All cells were cultured in 37°C with 5% CO₂.

Adenovirus infection and receptor profiling

Breast cancer cells were infected with the Ad-HA-GFP at staggered MOIs of 0, 10, 50, and 100 to controllably induce HA expression. Cells were trypsinized, aliquoted (250k cells/tube), and incubated with either biotinylated anti-HA antibodies (Sigma-Aldrich; St. Louis, MO) or biotinylated isotype control antibodies (SouthernBiotech, Birmingham, AL) at a concentration of 0.5 ug and 1.0 µg per 10⁶ cells, respectively. Cell groups were washed with cell buffer (PBS with 3% FBS) and centrifuged (Beckman Coulter, Brea, CA) to remove unbound antibodies. Cells were then incubated with a Cy5 labeled streptavidin (SouthernBiotech, Birmingham, AL) (0.5 µg per 10⁶ cells) followed by centrifugal washing. Receptor expression and assessment of antibody binding was quantified using flow cytometry methods (Accuri C6, Accuri Cytometers Inc, Ann Arbor, MI). Specifically, fluorescent counts were registered for a gated fixed cell count (10³ events). All studies were performed in triplicate.

Targeted contrast agent preparation and characterization

Targeted contrast agents were generated by conjugating biotinylated anti-HA antibodies to the biotin coated MB (Targestar-B, Targeson, San Diego, CA) surface via a streptavidin bridge. Streptavidin-coated MBs were incubated with anti-HA or isotype control antibody for 20 min followed by centrifugal washing. Final concentrations of targeted and control MB groups were characterized using a hemocytometer and reported as MBs/mL.

In vitro methods

2LMP Cells infected with staggered Ad vector MOIs of 0, 10, 50, and 100 were individually plated in 6-well dishes (Costar, Corning Inc, Lowell, MA) and incubated with 100 µL of either anti-HA targeted MBs or anti-isotype control MBs at a concentration of 1.3×10^7 MBs/mL. Plates were gently rocked (Boekel, Feasterville, PA) for 10 min. Plates were then washed in PBS to remove unbound MBs. Each cell group was imaged using brightfield or fluorescence mode microscopy (Olympus 1X70, Olympus America Inc, Melville, NY). Plates were evaluated by a blinded observer and the five most concentrated regions (40×, original magnification) were chosen for analysis. Closely aggregated MBs were able to be discerned at the 40× magnification. The number of cells and attached MBs within each field of view were recorded. Data was reported as attached MBs per cell. Each area was then imaged in fluorescence mode to confirm receptor expression via the GFP reporter.

Data analysis

Data recorded during the *in vitro* study was presented as the mean ± SEM. Fluorescent counts from flow cytometry data were graphed to visualize relationships between either cancer cell infection (GFP expression) or antibody binding to cell groups infected with

differing Ad vector MOIs. For Isotype and HA-targeted MB data sets, intra- and inter-group differences were assessed using a two-sample *t*-test. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

Characterization of induced reporter expression in breast cancer cells

Binding of anti-HA antibodies to cellular biomarkers expressed using Ad vector infection was demonstrated. A schematic of adenoviral infection and isotype/anti-HA targeted MB incubation is shown in Figure 1a–c. Incrementally increasing Ad vector MOI produced a corresponding increase in GFP expression as determined by optical detection and quantification of GFP reporter expression using flow cytometry (Figure 2). Ad-HA-GFP infected cells displayed progressively higher GFP expression with increasing Ad vector MOI ($p < 0.001$). Uninfected cells (MOI = 0) displayed no GFP expression.

HA receptor expression was also evaluated using flow cytometry techniques and results are shown in Figure 2. Regression analysis revealed a linear relationship between HA expression levels and Ad MOI ($r^2 > 0.96$). While no difference between anti-HA and anti-isotype antibody binding levels was seen at an MOI of 0 ($p > 0.29$), increasing the Ad vector MOI produced a corresponding increase in anti-HA antibody binding ($p < 0.01$). No binding trends were observed in the anti-isotype group data ($p > 0.44$) indicating that nonspecific antibody binding was minimal.

In vitro analysis of HA-targeted MB binding

Figure 3a–d depicts representative images (10× magnification) of HA-targeted MB binding of 2mp breast cancer cells infected with Ad-HA-GFP using staggered MOI. Regardless of HA expression level, no significant difference was found between MOI cell groups incubated with isotype-targeted MBs ($p > 0.42$). As shown in Figure 3e, very few isotype-targeted MBs were found to be attached (0.6 ± 0.3 MBs per cell). However, cells exposed to anti-HA labeled MBs showed significantly increasing levels of cellular binding proportional to HA expression levels ($p < 0.02$). The average number of HA-targeted MBs attached to cellular HA was 0.5 ± 0.3 MBs per cell when infected at an MOI of 0 (Figure 3a). However, for MOIs of 10 (Figure 3b), 50 (Figure 3c), and 100 (Figure 3d), the average number of attached MBs to infected cells was found to be 4.3 ± 0.5 , 6.1 ± 1.3 , and 12.3 ± 2.4 MBs per cell, respectively.

Fluorescent characterization of Ad infection of breast cancer cells

Successful Ad infection and receptor expression was verified by fluorescence imaging of the GFP reporter (Figure 4). Uninfected cells (Ad vector MOI of 0) showed no fluorescence signal and very little HA-targeted MB binding. Cells infected with an MOI of 10, 50, or 100 produced fluorescent signal intensity which corresponded to the HA expression as determined by HA-targeted MB binding. In addition, cells in the imaging frame with highest fluorescence showed enhanced HA-targeted MB binding. Variation in HA and GFP expression within the same MOI group is hypothesized to be due to cells in various stages of the cell cycle during Ad infection. The GFP expression shown in Figure 4 is directly proportional to the number of viral particles successfully infecting the cell, as confirmed by flow cytometry.

DISCUSSION

Reported here is a novel model for evaluating receptor-targeted MB binding using an Ad vector to modulate targeted receptor density. Controlled receptor expression was achieved

through the use of Ad gene transfer techniques (Zinn et al. 2002) and evaluated using flow cytometry. MB attachment to cellular receptors was characterized *in vitro* by modulating receptor expression followed by the addition of isotype-targeted and HA-targeting MB. Control and targeted US contrast agents were produced by conjugating antibodies to the MB lipid shell and exploiting biotin-streptavidin interactions.

For the *in vitro* studies, flow cytometry techniques were used to verify that cells were successfully infected with increasing Ad MOI resulting in dose-dependent reporter expression. Results showed a linear relationship between increased Ad dose and receptor expression density similar to what was reported in a previous study using the Ad-HA-GFP (Zinn et al. 2002). It is hypothesized that variation in the cell cycle plays an important role in transcription and translation of the adenovirus transgene. Additionally, it is common for some cells to be infected with less Ad vectors than others due to improper orientation of the Ad vector (Wu et al. 2004). Fewer particle infections in these cells would lead to fewer transcribed proteins unable to reach the threshold of expression. Importantly, results revealed that receptor expression was managed and modulation using different Ad-HA-GFP MOIs lead to a corresponding variance in anti-HA antibody binding, which was not observed using isotype control antibodies.

In vitro binding assays further illustrated that targeted MBs successfully adhere to cellular receptors and that attachment was proportional to receptor density. However, uninfected cells with no reporter expression showed only minimal MB attachment which was similar to control MBs. The presence of MBs in these groups could be attributed to nonspecific interactions or may simply be residual MBs from insufficient rinsing after incubation. Notwithstanding, differences in MB attachment between cells confirmed a selective binding and an affinity of targeted MBs for the target receptor. Additionally, results show that the receptor density directly correlates to the number of attached MBs. Previous *in vitro* studies have successfully used labeled MBs to target receptors such as P-selectin (Lindner et al. 2001; Warram et al. 2011), VEGFR2 (Willmann et al. 2008; Lyshchik et al. 2007; Warram et al. 2011), and alpha v integrins (Leong-Poi 2002; Ellegala et al. 2003; Warram et al. 2011) for adhesion to endothelial cell surfaces. In these studies, testing is restricted to a limited number of different cell lines and their intrinsic receptor expression levels. In order to correlate targeted MB binding to receptor density, different cell lines must be introduced to simulate receptor density variance. Conversely, the results from this study demonstrate that ligand density can be modulated within the same cell line using Ad vector techniques confirming that targeted MB binding is proportional to receptor expression. This strategy may prove to be useful for evaluating targeted MBs in applications such as disease staging, receptor profiling, or therapeutic monitoring. A limitation, however, of the *in vitro* experiments include that they were not performed with real-time flow conditions which would put the MBs under real-time shear stress. The number of attached targeted MBs will be affected with the varying wall shear rates, a critical parameter inside the human body (Weller et al. 2002). Future studies could include using the modulated receptor model under flow conditions utilizing a flow chamber and assessing MB binding with varying wall shear stress and Ad-MOI.

This model of induced receptor expression to evaluate targeted MB performance can be beneficial to both preclinical and clinical targeted MB research. During different stages of a progressive disease, varying levels of receptors are expressed at the disease site. Thus, since the results of this study provide a firm correlation between MB accumulation and receptor density, targeted MBs can be utilized to track specific markers during disease progression. Investigators from various disciplines can utilize Ad induced receptor expression to evaluate a variety of targeted MBs. Ad techniques can also be utilized to induce single versus multiple-targeted receptors to allow optimization of targeted MB performance.

Potential clinical benefit include using targeted MBs to track antiangiogenic therapies. Antiangiogenic therapy has been used to reduce vascularity to the tumor. Bevacizumab, an FDA approved monoclonal antibody against VEGF, in conjunction with chemotherapy has shown antiangiogenic activity and thus improve survival among metastatic cancer (Hurwitz et al. 2004; Miller et al. 2007). Targeted MBs can be used to assess the antiangiogenic response by characterizing MB accumulation throughout the therapy. Single targeted MBs will provide an opportunity to observe a specific receptor profile such as vascularity changes by targeting VEGFR. In contrast, multi-targeted MBs can provide a holistic approach for assessing antiangiogenic therapy by targeting multiple receptors to visualize changes within the tumor and its vascularity.

In conclusion, this study demonstrates use of Ad vector techniques for selectively controlling receptor expression in cancer cells. More importantly, modulation of receptor density results in a corresponding change in targeted MB accumulation at the receptor site. Ad induced receptors involved in angiogenesis, inflammation, and cardiac disease can be used to optimize targeted MB performance allowing investigators to evaluate possible target receptors associated with these conditions. Overall, contrast-enhanced US using controlled receptor-targeted MBs resulted in cellular MB accumulation which was dependent upon induced receptor expression.

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REFERENCES

- Anderson CR, Rychak JJ, Backer M, Backer J, Ley K, Klivanov AL. scVEGF microbubble ultrasound contrast agents: a novel probe for ultrasound molecular imaging of tumor angiogenesis. *Invest Radiol.* 2010; 45:579–585. [PubMed: 20733505]
- Behm CZ, Kaufmann BA, Carr C, Lankford M, Sanders JM, Rose CE, Kaul S, Lindner JR. Molecular imaging of endothelial vascular cell adhesion molecule-1 expression and inflammatory cell recruitment during vasculogenesis and ischemia-mediated arteriogenesis. *Circulation.* 2008; 117:2902–2911. [PubMed: 18506006]
- Christiansen JP, Leong-Poi H, Klivanov AL, Kaul S, Lindner JR. Noninvasive imaging of myocardial reperfusion injury using leukocyte-targeted contrast echocardiography. *Circulation.* 2002; 105:1764–1767. [PubMed: 11956115]
- Ellegala DB, Leong-Poi H, Carpenter JE, Klivanov AL, Kaul S, Shaffrey ME, Sklenar J, Lindner JR. Imaging tumor angiogenesis with contrast ultrasound and microbubbles targeted to $\alpha v \beta_3$. *Circulation.* 2003; 108:336–341. [PubMed: 12835208]
- Floyd E, McShane TM. Development and use of biomarkers in oncology drug development. *Toxicol Pathol.* 2004; 32:106–115. [PubMed: 15209410]
- Friedlander M, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, Cheresch DA. Definition of two angiogenic pathways by distinct α -v integrins. *Science.* 1995; 270:1500–1502. [PubMed: 7491498]
- Hurwitz H, Fehrenbacher L, Novotny W, Catwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffin S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus Irinotecan, Fluorouracil, and Leucovorin for metastatic colorectal cancer. *N Engl J Med.* 2004; 350:2335–2342. [PubMed: 15175435]
- Kaufmann BA, Lindner JR. Molecular imaging with targeted contrast ultrasound. *Curr Op in Biotech.* 2007; 18:11–16.
- Kaufmann BA, Carr CL, Belcick JT, Xie A, Yue Q, Chadderdon S, Caplan ES, Khangura J, Bullens S, Bunting S, Lindner JR. Molecular imaging of the initial inflammatory response in atherosclerosis: implications for early detection of disease. *Atheroscler Thromb Vasc Bio.* 2009; 30:54–59.

- Klibanov AL. Ligand –carrying gas-filled microbubbles: Ultrasound contrast agents for targeted molecular imaging. *Bioconjugate Chem.* 2005; 16:9–17.
- Klibanov AL, Rychak JJ, Yang WC, Alikhani S, Li B, Acton S, Lindner JR, Ley K, Kaul S. Targeted ultrasound contrast agent for molecular imaging of inflammation in high-shear flow. *Contrast Media Mol Imaging.* 2006; 1:259–266. [PubMed: 17191766]
- Knowles JA, Heath CH, Saini R, Umphrey H, Zinn KR, Hoyt K. Molecular targeting of ultrasound contrast agent enhances detection of head and neck squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg.* 2011 (Submitted for publication).
- Korpanty G, Carbon JG, Grayburn PA, Fleming JB, Brekken RA. Monitoring response to anticancer therapy by targeting microbubbles to tumor vasculature. *Clin Cancer Res.* 2007; 13:323. [PubMed: 17200371]
- Leighton, TG. *The Acoustic Bubble.* London: Academic Press; 1997.
- Leong -Poi H, Christiansen J, Klibanov AL, Kaul S, Lindner JR. Noninvasive assessment of angiogenesis by ultrasound and microbubbles targeted to alpha-v integrins. *Circulation.* 2002; 107:455–460. [PubMed: 12551871]
- Lindner JR, Song J, Christiansen J, Klibanov AL, Xu F, Ley K. Ultrasound assessment of inflammation and renal tissue injury with microbubbles targeted to P-selectin. *Circulation.* 2001; 104:2107–2112. [PubMed: 11673354]
- Lindner JR. Microbubbles in medical imaging: current applications and future directions. *Nature Reviews.* 2004; 4:527–532.
- Lindner JR. Contrast ultrasound molecular imaging of inflammation in cardiovascular disease. *Cardiovasc Res.* 2009; 84:182–189. [PubMed: 19783842]
- Lyshchik A, Fleisher AC, Huamani J, Hallahan DE, Brissova M, Gore JC. Molecular Imaging of vascular endothelial growth factor receptor 2 expression using targeted contrast-enhanced high-frequency ultrasonography. *J Ultrasound Med.* 2007; 26:1575–1586. [PubMed: 17957052]
- Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, Shenkier T, Cella D, Davidson NE. Paclitaxel plus Bevacizumab versus Paclitaxel alone for metastatic breast cancer. *N Engl J Med.* 2007; 357:2666–2676. [PubMed: 18160686]
- Unger EC, McCreery TP, Sweitzer RH, Shen D, Wu G. *In vitro* studies of a new thrombus-specific ultrasound contrast agent. *Am J Cardiol.* 1998; 81:58–61.
- Warram JM, Sorace AG, Saini R, Umphrey H, Zinn KR, Hoyt K. Triple-targeted US contrast agent provides improved localization to tumor vasculature. *J Ultrasound Med.* 2011 (In press).
- Weller GE, Villanueva FS, Klibanov AL, Wagner WR. Modulating targeted adhesion of an ultrasound contrast agent to dysfunctional endothelium. *Ann of Biomed Eng.* 2002; 30:1012–1019. [PubMed: 12449762]
- Willmann JK, Paulmurugan R, Chen K, Gheysens O, Rodriguez-Porcel M, Lutz AM, Chen IY, Chen X, Gambhir SS. US imaging of tumor angiogenesis with microbubbles targeted to vascular endothelial growth factor receptor type 2 in mice. *Radiology.* 2008; 246:508–518. [PubMed: 18180339]
- Willmann JK, Kimura RH, Deshpande N, Lutz AM, Cochran JR, Gambhir SS. Targeted contrast-enhanced ultrasound imaging of tumor angiogenesis with contrast microbubbles conjugated to integrin-binding knottin peptides. *J of Nuclear Med.* 2010; 51:433–440.
- Wu E, Nemerow GR. Virus yoga: the role of flexibility in virus host cell recognition. *Trends Microbiol.* 2004; 12:162–169. [PubMed: 15051066]
- Zinn KR, Chaudhuri TR, Krasnykh VN, Buchsbaum DJ, Belousova N, Grizzle WE, Curlel DT, Rogers BE. Gamma camera dual imaging with a somatostatin receptor and thymidine kinase after gene transfer with a bicistronic adenovirus in mice. *Radiology.* 2002; 223:417–425. [PubMed: 11997547]

Figure 1.

(a) Basic schematic diagram of the adenoviral infection which induces HA receptor expression with a GFP tag. (b) Diagram of anti-isotype MBs incubated in a cell layer. Few of the MBs illustrate non-specific binding to the cell layer. (c) Depiction of anti-HA targeted MBs firmly attaching to the ad-induced HA receptors.

Figure 2.

Modulating Ad-HA-GFP infection leads to corresponding GFP and HA expression in breast cancer cells. 2LMP breast cancer cells were infected at MOIs of 0, 10, 50, and 100. Cells were then evaluated for GFP and HA expression using flow cytometry. Cy-5 conjugated antibodies against HA and HA antibody isotype were used. Data are means $\pm$ SE.

Figure 3. Increasing MOI resulted in higher MB adsorbed to the cellular receptors. Optical microscopy images (40 $\times$, original magnification) of breast cancer cells (2LMP) after infection with an Ad-HA-GFP at an MOI of (a) 0, (b) 10, (c) 50, and (d) 100. (e) In vitro binding assay with Ad infected 2LMP cells incubated with anti-HA MB. Data are means $\pm$ SE.

Figure 4. Increased receptor expression produces a corresponding increase in MBs adhered to the cell surface which corresponds to GFP reporter expression. Optical (left) and fluorescence (right) microscopy images of HA-targeted MB binding and GFP reporter expression levels, respectively.