Multimodal Imaging-Based Characterization of HER2+ and Triple Negative Metastatic Breast Cancer Xenograft Models in Mice

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ABSTRACT SUMMARY

Two metastatic breast cancer xenograft models useful for assessment of HER2 targeted therapies were successfully characterized longitudinally using a multimodal pre-clinical imaging approach.

INTRODUCTION

Novel drug delivery systems for anticancer therapies have been commonly assessed in xenograft models of human cancers in mice. These models are limited in their ability to replicate the clinical presentation of disease. In particular, there is a need for more relevant models that recapitulate metastatic patterns observed clinically. Employing such models presents challenges associated with monitoring of metastasis in vivo. Thus, upon development of such a model, full characterization is imperative to identify adequate methods for metastasis monitoring and to determine expected metastatic sites and their spatial and temporal characteristics. This knowledge allows for appropriate design of experiments that aim to assess novel therapeutics in metastatic cancers. In this study, HER2+ and triple negative (TN) metastatic breast cancer xenograft models in mice were characterized using non-invasive multimodality imaging for visualization, localization and characterization of primary and metastatic lesions. These models are suitable for clinically relevant assessment of HER2-targeted therapeutic delivery systems. The imaging approach presented here is shown to be useful in the performance assessment of nano-sized drug delivery systems.

EXPERIMENTAL METHODS

LM2-4 is a highly metastatic variant of the TN MDA-MB-231 human breast cancer cell line, developed through two rounds of spontaneous lung metastasis selections in mice¹. LM2-4 cells were transduced with erbB2 to achieve the HER2+ LM2-4H2N cell line ¹,². Female SCID mice were inoculated with 50µL of 2 or 4x10⁶ LM2-4 or LM2-4H2N cells into their right inguinal mammary fat pad (MFP). Primary tumor volumes were quantified using preclinical micro-computed tomography (µCT, Locus Ultra, GE) and were surgically resected 2 weeks post-inoculation (250-400 mm³). As both cell lines express luciferase, bioluminescence imaging (BLI) was performed longitudinally to monitor primary and metastatic tumor development. Mice were injected i.p. with 150mg/kg D-luciferin (Caliper Life Sciences) 10 minutes prior to BLI acquisition. Signal was detected using an IVIS System (Xenogen, 5-10 second acquisition). To compare the metastatic potential of these cell lines to the parental TN MDA-MB-231, a luciferase-expressing version of this line was employed (ATCC). The same inoculation protocol was used, and primary tumors were removed upon reaching a volume of 250-400 mm³. At 48 hours prior to the study endpoint, a CT/optical liposomal dual-modality contrast agent was administered i.v. for qualitative assessment of nano-system delivery. In vivo contrast-enhanced µCT images were acquired, and ex vivo 2-D near-infrared (NIR) fluorescence visualization of metastatic lesions was performed using the Maestro system (Perkin Elmer).
RESULTS AND DISCUSSION

All mice developed primary tumors in the inguinal MFP. Inoculation of LM2-4 and LM2-4H2N with \(2 \times 10^6\) cells resulted in variable tumor growth. Primary tumors reached the target range of 200-400 mm\(^3\) within a broad timeframe (days 14-36). Mice inoculated with \(4 \times 10^6\) cells reached an average primary tumor volume of \(262.62 \pm 79.53\) mm\(^3\) (\(n = 10/\text{model}\)) on day 14, at which point tumors were surgically resected. Metastatic lesions were detected by BLI between days 18-28 (LM2-4) and days 24-35 (LM2-4H2N) post-inoculation. Metastatic incidences of the LM2-4 and LM2-4H2N models were 89\% (8/9) and 78\% (7/9), respectively. Sites of metastasis (peritoneum, liver, lung and other MFPs) were consistent with previously published reports\(^1\) and represent the clinical presentation of advanced-stage breast cancer\(^1\). The metastatic incidence of the MDA-MB-231 model was only 40\% (2/5).

Primary tumors were visualized by BLI and \(\mu\)CT. Tumor volumes determined by \(\mu\)CT correlated with caliper-based measurements (\(R^2 = 0.76\)). At the study endpoint, 48 hours post-injection of the CT/optical liposome agent, contrast-enhanced \(\mu\)CT allowed for identification and quantification of minute metastatic lesions as small as 2.2 mm\(^3\) not identifiable by BLI. Further, the high spatial resolution of \(\mu\)CT allowed for delineation of individual lesions found in the same vicinity that appeared as one large lesion in BLI images (Figure 1).

\[\text{Figure 1. Lesions in the same vicinity appear as one cluster in BLI (left). Contrast-enhanced } \mu\text{CT allows for resolution of individual lesions.}\]

\[\text{In vivo BLI allowed for temporal and spatial assessment of metastatic tumors. Ex vivo NIR fluorescence imaging allowed for ready identification of lesions in challenging sites such as the lungs (Figure 2), which were pathologically confirmed as tumorigenic.}\]

\[\text{Figure 2. } \text{Ex vivo NIR fluorescence imaging (A, lower panel) confirmed the presence of a lung lesion (A, upper panel) seen by contrast-enhanced } \mu\text{CT (B) and not detected by BLI (C). The lesion was confirmed pathologically (D).}\]

CONCLUSION

This work presents comprehensive imaging-based characterization of the metastatic attributes of two xenograft models of metastatic breast cancer, LM2-4 and LM2-4H2N. As these models are similar in their metastatic incidences, sites and tumor growth rates, but differ in HER2 positivity, they are useful for the assessment of therapeutic delivery systems targeted to HER2.

Results obtained here demonstrate the feasibility of employing BLI, NIR fluorescence and \(\mu\)CT imaging to characterize metastatic models, to monitor metastasis progression over time, and to assess performance of nano-sized delivery systems. The combination of these imaging techniques allowed for non-invasive, accurate, real-time tracking of metastasis, overcoming limitations of endpoint-based studies.

REFERENCES


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