Imaging Cytokine Targeting to the Tumor/Bone Microenvironment *In Vivo*

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FIG. 1. Gaussia luciferase modified at the C-terminus with tumor or bone targeting peptides localize to intratibial prostate tumors. (A) Sonodelivery schematic and muscle secretion of tumor-targeted Gluc cytokine model. (B) TC2R-*Luc*-marked prostate tumor cells implanted in tibia on day 0 give a detectable signal by day 3. Plasmids expressing Gluc (pGL) are delivered intramuscularly (I.M.) via sonodelivery on day 4, and mice imaged for GLuc expression at day 7 (C). (D) *Ex vivo* imaging on day 7 shows that only targeted pGluc show signals in tumor/bone metastases. (E) Quantification of fold GLuc signal over control in tumor/bone versus normal organs on day 7 or 18 postsonoporation. In addition, * $p < 0.04$ compared with normal organs; # $p < 0.03$ Tu/bone on day 18 compared with Tu/bone signals on day 7. Ctrl refers to delivery of pGL, a plasmid expressing Gaussia luciferase bearing *Gluc* with a scrambled, nonspecific peptide sequence. Color images available online at www.liebertpub.com/humc

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A MAJOR HURDLE OF SYSTEMIC cytokine therapies is achieving therapeutic levels within the affected tissue, especially in the case of bone metastatic cancer. For instance, bone is the most common site for castrate-resistant prostate cancer metastasis, and the majority of patients with advanced disease will develop painful bone metastases and fractures, with a reduced quality of life and poor prognosis. Bone metastatic tumors are very difficult to treat since there may be multiple metastatic sites, and these may not be accessible to surgical or radiation interventions. Cytokine immunotherapies hold great promise since these are secreted molecules that can reach and treat distant bone metastatic tumors. Importantly, there remains a lack of therapeutics that can simultaneously and effectively treat the prostate tumor while restoring affected bone tissue. Our lab has developed several cytokine therapeutics specifically for treating the tumor/bone microenvironment.

In order to deliver cytokines efficiently to tumor/bone metastases, we have devised a strategy based on C-terminal modification of secreted molecules for enabling their targeting and accumulation at metastatic sites. However, there are no current systems for measuring the delivery, binding, and accumulation of cytokines at the complex tumor/bone microenvironment. The present study thus examined the potential development of a reporter molecule or “cytokine model,” using a secreted luciferase (Gaussia Luc or GLuc), which we hypothesized would mimic therapeutic cytokine secretion, targeting, and accumulation in tumors.

We used the gene delivery system called “sonodelivery” to deliver plasmid DNA (pDNA) to skeletal muscle of male mice bearing intratibial prostate tumors. Sonodelivery utilizes a novel oligolysine polymer (Parelkar *et al.*, 2014) complexed to pDNA to create a nanoplex that is delivered along with microbubbles and sonicated to achieve ultrasound-enhanced muscle transfection (Fig. 1A) (Zolochovska *et al.*, 2011, 2013).

Using a tumor/bone metastasis model (Zolochovska *et al.*, 2013), we labeled TRAMPC2Ras (TC2R) prostate tumor cells with firefly luciferase (FLuc) to show localization of tumor cells with luciferin substrate (Fig. 1B). Next, we sonodelivered a plasmid expressing GLuc to the hind thigh muscle. Seven days following sonodelivery, we detected accumulation of peptide-targeted but not wild-type GLuc at the tumor/bone metastatic sites when using coelenterazine substrate (Fig. 1C). The peptide-targeted GLuc were modified at the C-terminus by addition of peptide sequences that could specifically bind receptors upregulated in tumor (or remodeling bone) but not in other tissues. The peptides we have chosen bind IL6-R α [pepTu; *LSLITRL* (Su *et al.*, 2005)] or BMPR1b [pepB; *AISMLYLDENEKVVL* (Lin *et al.*, 2005)] and thus are relevant for targeting therapeutic cytokines for tumor/bone retention. We used computational design to identify an optimal linker, 1x(Gly₄Ser), between

GLuc and the peptide to enhance the likelihood of native peptide folding, and showed improved Gluc binding up to 100-fold in tumor cells. These peptide-targeted GLuc retained functional activity and were able to localize and accumulate Gluc at intratibial tumors following sonodelivery at levels up to 123-fold higher than the control (Fig. 1D and E). Therefore, we present here the first data, to our knowledge, of utilizing GLuc as a cytokine model to assess targeting of C-terminal peptides to the prostate tumor/bone microenvironment *in vivo*.

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