Carestream Molecular Imaging: imaging of cancer biology and relevant pathways *in vivo*

Here we highlight recent published advances in fluorescent reporters that use the Carestream Molecular Imaging systems for studying cancer biology pathways *in vivo*. In particular, we highlight reporters that allow the rapid assessment of relative protein expression levels, cell migration and tumor morphology.

In vivo imaging, whether at the cellular or whole-animal level, promised to revolutionize preclinical and clinical biological and bioengineering research. By conducting longitudinal studies, the natural course of a disease or the experimental model can be monitored. To gain molecular information in cross-sectional studies, animals are dissected and studied via techniques such as immunoblotting (western blot), immunofluorescence, immunohistochemistry, quantitative real-time RT-PCR, microarray, high-throughput sequencing or fluorescence-activated cell sorting (FACS) analysis. However, these techniques provide only information at the time of measurement, and they often require animals to be euthanized. With *in vivo* imaging, not only is the true time course in each animal observed, but spatiotemporal information can better guide the use of classical molecular biology techniques. Carestream Molecular Imaging offers the In Vivo Multispectral FX package, which includes a combined high-resolution optical and X-ray imaging instrument and software analysis package. The software package offers excitation-based spectral unmixing with direct co-registration with X-ray and reflectance images. Other instrument configurations available include both automated and manual formats, and all of the automated systems can be upgraded to the In Vivo Multispectral FX without purchasing an entirely new instrument.

In vivo imaging of relative protein expression level

In vitro assays such as immunoblotting (western blots) are commonly used to quantify relative protein expression. Optical probes can be used for *in vivo* 'westerns' for cell surface proteins. The VEGF (vascular endothelial growth factor) pathway is an area of active study for tumorigenesis and metastatic potential of tumors. Understanding and quantifying the relative changes in VEGF receptor (VEGFR) at a tumor site could yield insight into the relevance of various tumor models, as well as the ability of potential therapeutics to block the binding site of this receptor. Fluorescently labeled VEGF-B has been used to visualize VEGFR expression at tumor sites by co-registering optical signals at the tumor site using the Carestream Molecular Imaging platform[1]. This approach is superior to traditional western blotting techniques for studying VEGFR expression because therapeutics and biologics that either change expression levels or block the VEGF binding site can be readily elucidated using this methodology.

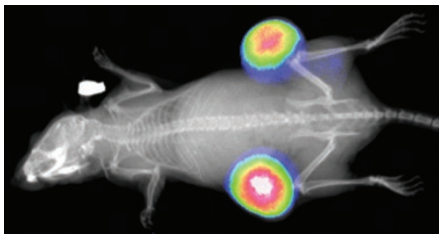

Figure 1 | Tumor targeting by fluorescently labeled antibody. A nude mouse was implanted with tumors known to bear a specific antigen. These mice were injected with near-infrared fluorophore–labeled antibodies targeting this antigen. Mice were imaged 24 hours after antibody injection on the In Vivo Multispectral FX (Carestream Molecular Imaging) in fluorescence and X-ray mode for simple co-registration. Antigen was detected at the tumor site. Figure courtesy of Abraxis Bioscience.

In a direct analogy to western blotting, fluorescently labeled primary antibodies can be used to analyze protein expression *in vivo* (**Fig. 1**). Antibodies can be labeled with a variety of dyes, although those such as Carestream Molecular Imaging's X-Sight 761 and LSS 670 are optimal for *in vivo* imaging owing to their excitation and emission in the near infrared. To facilitate the work in labs that do not have access to bioconjugation chemists, Carestream Molecular Imaging can custom-label any primary or secondary antibody provided by researchers with either of these dyes. Antibodies have long circulation times *in vivo* and long residence times at the target site, limiting the time resolution of these experiments to 24–48 hours[2]. Yet the ease of transition from traditional *ex vivo* techniques to *in vivo* imaging with antibodies may more than make up for this limitation.

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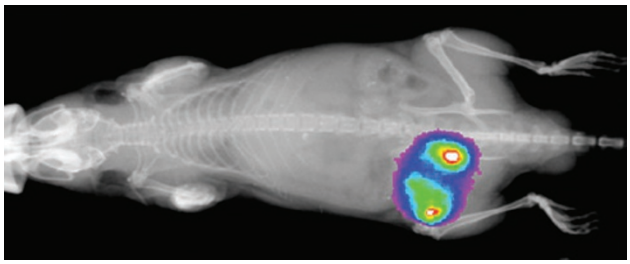

Figure 2 | Tumor imaging by prelabeling cells with near-infrared nanoparticles. Melanoma cells were prelabeled with Kodak X-SIGHT 761 (Carestream Molecular Imaging) nanoparticles and subsequently imaged in the In Vivo Multispectral FX. Multiple foci of growth are apparent.

The availability of receptors and receptor binding sites has long been studied in the nuclear medicine field and now can be approached by optical methods as well. Indeed, $\alpha_v\beta_3$ integrins have been imaged in the Carestream Molecular Imaging In Vivo Imaging System FX by researchers using near-infrared fluorophore–labeled peptides[3]. In addition, after high-throughput screening of compounds, a biotinylated peptide that targets $\alpha_4\beta_1$ integrin has also been developed and used to image these receptors *in vivo*[4]. When preconjugated to a near-infrared fluorophore–labeled streptavidin, this probe was able to detect these receptors specifically *in vivo*. The μPA receptor has recently been imaged on the surface of tumors in live animals by a combination of optical imaging with X-ray co-registration as well as subsequent imaging by magnetic resonance imaging (MRI) and fluorescence microscopy[5]. Thus, if there is a specific peptide available for a receptor it often can be labeled with a near-infrared fluorophore and the receptor availability tracked *in vivo*.

Imaging protein activity *in vivo*

Beyond the presence or absence of a protein or molecular substrate, the enzymatic activity of a given protein may be of paramount importance. The ability of pathways and small molecules to activate or inhibit the caspase pathway can literally mean the difference between life and death, for a patient or an individual cell. Simply detecting the presence of caspase-3 would not be sufficient, as it is constitutively expressed in a wide variety of cells. Only upon proteolytic cleavage does the caspase become active. Thus a probe to optically image caspase activity directly would have a great deal of utility. A quenched, near-infrared–emitting probe was developed that upon caspase activation yields an active fluorophore[6]. After activation, the fluorophore is released from the quencher and trapped intracellularly, yielding a bright fluorescent signal at sites of caspase activity. The *in vivo* optimization and characterization of this probe was executed on the Carestream Molecular Imaging In Vivo Imaging System FX. Using the same system, others have shown proof-of-principle *in vivo* of a dual fluorescence and radioisotope probe for *in vivo* imaging of caspase activity, but still need to make this probe available intracellularly to reach its full potential for monitoring caspase activity *in vivo*[7]. Both of these designs are modular and could be applied to a wide variety of proteases for detecting their activity.

Imaging tumor localization and morphology

Cells have been known to silence genetically encoded reporters. For these cell lines or for researchers who do not wish to disturb the genome of their tumor cells, prelabeling tumor cells to track their localization and morphology *in vivo* present an exciting opportunity. Indeed, X-SIGHT 761 nanoparticles (Carestream Molecular Imaging) have been demonstrated to label cells for *in vivo* tracking for more than a week, and these methods require no manipulation of the genome of the cells (**Fig. 2**). Because X-SIGHT 761 particles both excite and emit in the near infrared, they provide maximum sensitivity by *in vivo* fluorescence imaging.

Conclusion

In vivo imaging of fluorescent probes allows the rapid, nondestructive imaging of processes and pathways relevant to cancer biology. Carestream Health's In Vivo MS FX and In Vivo Imaging product line provides a robust platform for high-resolution optical imaging of these pathways and allows the capture of phenotypic and co-registration information via our high-resolution imaging systems.

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