Whole body optical imaging in small animals and its translation to the clinic: Intra-operative optical imaging guided surgery

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Abstract

Whole body optical imaging using bioluminescence or fluorescence is one of the most rapidly emerging technologies to non-invasively follow all kinds of molecular and cellular processes in small animals. Using tomographic approaches it is now also possible to get better quantitative data. Due to its sensitivity and simplicity it is now also widely used in drug development and drug screening. Finally, using near infrared fluorescent probes that have much deeper penetration also opens up new exciting applications such as intra-operative image guided surgery for sentinel lymph node mapping and radical resection of tumours.

Recent advances in imaging strategies that reveal cellular and molecular biological events in real-time facilitate our understanding of biological processes occurring in living animals. The development of molecular tags, such as green fluorescent protein (GFP) from the jellyfish Aequorea victoria, red fluorescent proteins (RFP) from the Discosoma species (dsRed2) and luciferase (Luc) from the firefly Photinus pyralis (fLuc) and the sea pansy Renilla (rLuc), has revolutionised research over the past decade, allowing complex biochemical processes to be associated with the functioning of proteins in living cells.

Optical technologies, both microscopic and macroscopic, are developing fast. Recent technical advances for imaging weak visible light sources using cooled charged coupled device (CCCD) cameras, peltier cooled detectors and micro-plate channel intensifiers allow detection of photon emission from inside the tissues of small animals. Whole body **fluorescent imaging (FLI)** and **bioluminescent imaging (BLI)** are now applied to study cell- and tissue-specific gene promoter activity and also to follow trafficking, differentiation and fate of i.e. GFP or RFP and/or luciferase expressing cells, or biological processes like apoptosis, protein–protein interaction, angiogenesis, proteolysis and gene-transfer. Optical imaging (OI) and optical reporter systems are also very cost-effective and time-efficient and they are particularly well suited for small animal imaging and for *in vitro* assays to validate different reporter systems.

Whole body BLI

Until recently, BLI was the most commonly used technology for whole body optical imaging since there are several important advantages of using firefly luciferase as a reporter. The fLuc protein, in contrast to other reporter proteins, has a relatively short half-life of about 1.5 h. This feature makes it ideally suited for kinetic and dynamic analysis of gene expression within short time frames and, therefore, to identify circadian or even infradian rhythms of gene expression. There is no background activity, making it very specific and sensitive. This is in contrast to GFP signals where endogenous autofluorescence of tissues frequently results in substantial background emissions that until recently has limited the sensitivity and specificity of this imaging technique. This has contributed to an important advantage of bioluminescence over fluorescence reporters. However, the use of selective filters and the application of spectral analysis can significantly reduce the contribution of autofluorescence to the acquired images.

Whole body FLI

Limitations of fluorescence GFP reporter imaging include the requirement of an external source of light and the exponentially decreasing intensity of light with increasing depth of the target. However, a new class of red fluorescent proteins and its more red shifted variants mRaspberry, mCherry, mPlum and

Katushka, as well as the development of near-infrared dyes and quantum dots that can be coupled to all kinds of ligands, antibodies etc., are providing better deep tissue imaging characteristics (penetration of cm's). These new developments have made whole body FLI an interesting additional technology besides whole body BLI.

General applications in cancer

In Cancer Research whole body optical imaging has allowed semi-quantitative measurements of tumour progression and metastasis and treatment response. Due to its high sensitivity FLI, and especially BLI, are extremely useful for early detection of micro-metastases and minimal residual disease states in animal models. Apart from using "smart" gene-reporters, the use of "smart" injectable near infrared fluorescent (NIRF) probes or quantum dots linked to all kinds of ligands, compounds, antibodies etc., also makes it possible to non-invasively follow molecular processes like proteolysis, bone turnover, apoptosis and angiogenesis. These processes play an important role in cancer but also in tissue remodelling in general. Using OI specific gene-reporters either in transplanted tumour cells or in transgenic gene-reporter animals allows us to study gene-expression, -regulation and -function, also known as Functional Genomics. OI is also perfectly suited to more rapidly develop and screen the efficacy of new drugs and therapies. Finally, using highly specific antibodies, like very small single chain lama-antibodies (nanobodies), or Affibodies, labelled with near infrared fluorescent probes, FLI can now also be used in image-guided surgery for cancer.

Going from 2D qualitative to 3D quantitative whole body optical imaging

Optical imaging has been based on 2D planar images and, therefore, spatial resolution was poor and quantification difficult and at best semi-quantitative. New developments have now made it possible to extend FLI and BLI to three-dimensional imaging by 3D optical tomography allowing better quantification of photon emission. In addition, fusing 3D optical images with images obtained from the same animal using magnetic resonance imaging (MRI) or fast computed tomography (CT) will allow structural anatomic information to be obtained and will greatly enhance spatial resolution. Furthermore, structural tissue information obtained by fast CT or MRI will also allow generation of a tissue atlas that can be used to correct for tissue-dependent photon scattering and absorption. This now allows better quantitative data to be obtained.

Intra-operative optical imaging guided surgery

Using non-invasive imaging technologies like CT, MRI and positron emission tomography (PET), cancer can be detected much earlier and the tumour can subsequently be removed using minimal-invasive techniques like endoscopy or laparoscopy or by open surgery. However, it is of great importance that during operation the tumour is radically removed with enough tumour-free margins. Because it is difficult to distinguish between tumour tissue and normal tissue during surgery, it is also difficult to determine an adequate tumour-free margin, making non-radical resections a serious clinical problem. It is still also a problem to identify sentinel lymph nodes or local (lymph node) metastases during surgery. However, if during operation one is able to identify tumour margins, sentinel lymph nodes and local metastases using optical imaging and subsequently radically remove them, the life expectancy of cancer patients will greatly improve. A promising development in this area is the use of NIRF imaging. For this, NIRF molecules are coupled to proteins, i.e. antibodies, or other drugs that will specifically bind to the tumour, making real-time visualisation of tumour tissue during operation possible. Recently, enzyme activatable NIRF probes have also become available that are specifically cleaved by enzymes like MMPs (MMPsense^{TM}) or Cathepsins (Prosense^{TM}) that are up regulated in the invasive front of many tumours. In this way, tumour cells in the cutting edge or in lymph nodes can specifically be made visible with high sensitivity.

In the current presentation, we will show examples of how tumour progression and metastasis, angiogenesis, epidermal growth factor receptor status and proteolysis can be monitored non-invasively in mice using whole body optical imaging. Finally, we will also show how, during operation, near infrared probes can be used for sentinel lymph node mapping and for radical tumour resection in mice and rats. In conclusion, we think that intra-operative optical imaging guided surgery using new "smart" NIRF probes is an attractive new tool in the field of surgical oncology.

Conflict of interest statement

None declared.

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