LISTENING TO REPORTER PROTEINS: HOW LOUD DOES THE MESSAGE NEED TO BE?

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Introduction. Optical imaging as non-invasive modality has tremendous research applications in the area of biomedical sciences such as characterization of cancerous cells. However, this imaging modality is limited by depth of light penetration of around 1 mm in living tissues obscuring visualization in vivo. Optoacoustic imaging is a potential solution of this problem based on detection of ultrasound produced by light-absorbing molecules exposed to laser radiation resulting in a tissue contrast. The image contrast relies on absorption of laser emission, however providing ultrasound resolution in living tissues. This study characterized properties of colorectal adenocarcinoma cells expressing Near-infrared Fluorescent proteins (iRFPs) for detection and visualization in Multispectral Optoacoustic Tomography (MSOT) settings in both tissue-mimicking phantoms and mice. We estimated variables affecting MSOT imaging of 3D multicellular tissue spheroids such as size, expression of iRFP in vitro. We tested MSOT for detection of subcutaneously implanted tumours expressing iRFPs in BALB/C nude mice *in vivo*.

Materials and methods. Human colorectal adenocarcinoma HT-29, HT-29 cell line expressing iRFP702 and a HT-29 cell line expressing iRFP713 were used for experiments. Fluorescence at excitation maximum of 645 nm and emission maximum of 710 nm was recorded. Induction of 3D tissue spheroids was done in 96-well ultra-low attachment Corning® plates. Spheroids were imaged with a light microscope connected to a digital camera at day 3. ImageJ software was used to assess sizes of spheroids. Tissue -mimicking phantoms were prepared by mixing agar with nigrosin ink and Intralipid solution. The resulting composition had optical density around 0.03 at 800 nm absorption. MSOT inVision 256-TF small animal scanner (iThera Medical GmbH, Munich, Germany) was used for multispectral image acquisition in the range of 600 - 850 nm. BALB/C mice underwent subcutaneous implantation of above-mentioned cell lines ($5*10^6$ cells suspended in 100 **Ц1** bilaterally).

Results and discussion. We showed that fluorescent spectral unmixing of iRFP702/ 713 expressing cells was possible for 10^7, 10^6, and 10^5 cells. We confirmed iRFP702 expression in both 3D and 2D cell cultures. Spheroids were increasing in size in positive correlation with number of cells used for initiation during a 3-day period. Qualitative image analysis confirmed detection of iRFP-expressing spheroid embedded into tissue-mimicking phantoms. However, quantitative data must be processed and analyzed to check whether the system differentiates between iRFP-expressing spheroids and control spheroids. Tumors originated from subcutaneous injections were identified via MSOT. iRFP contrast was distributed throughout the body of mice with partial coverage of iRFP702 and iRFP713 expressing tumors.

Conclusions. We developed 3D tumour models (spheroids) for MSOT analysis in vitro. We confirmed iRFPs expression in spheroids, tumours and fluorescent spectral unmixing in monolayer cultures. The next steps will involve studies of MSOT imaging of spheroids of controlled size, expression level, number of viable cells to identify a minimal number of cancer cells detectable with the MSOT imaging system

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