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In vivo FRET-FLIM imaging of apoptosis commitment in metastasized colorectal tumor cells

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We have developed an assay which allows fluorescent microscopical quantification of apoptosis commitment in metastasized colorectal tumor cells in response to anticancer drug treatment at different time points in living mice. The assay is based on the ability of activated Caspase-3 to cleave the tHcred1-DEVD-EGFP construct specifically, thus eliminating a FRET, an effect, which can be detected by Fluorescence Lifetime Imaging microscopy. For the first time a FLIM sensor has been employed in an animal model.

In conclusion we can make the following statements:

1. The tHcred1-DEVD-EGFP sensor demonstrates a reliable performance for apoptosis detection and quantification in adherent live C26 cells under cell culture conditions. The sensor can monitor the course of apoptosis in a single live cell at real time, without performing any of harvesting, fixation, permeabilisation and staining procedures. This helps to circumvent any involvement of side-effects of staining and fixation agents.

2. The tHcred1-DEVD-EGFP sensor executes functionality in C26 cells metastasized to the peritoneal cavity and liver, which allows to monitor the Caspase-3 activity in fresh mice tumour tissue by FLIM, thereby permitting to identify apoptotic cells. Furthermore, the model allows absolute quantification of FRET in a tumor animal model, which is another novelty of this work.

3. The Caspase-3 sensor allowes to monitor the dynamics of the development of tumor drug-resistance to one particular treatment. The assay demonstrates when and how chemoresistance progresses. Currently, the resistant cells provide a platform for extensive expression studies which might bring insight into resistance mechanisms.

4. Inhibition of survival mechanisms by RTK inhibitors seem to be applicable in the case of 5-FU resistancy.

5. The system can also be used to study other proteases in live cells by replacing the DEVD cleavage site by other specific recognition sequences.

6. It is easily conceivable to transfer the model of tHcred1-DEVD-EGFP transfected C-26 tumor cells to tumor cells cultured from biopsy specimens of cancer patients which are then – after adenoviral transfection – implanted into immunocompromised mice. Using the same technical platform, such a model could also be transferred to other cancer diseases and to monitor cancer drug therapies and allow the modeling of a specific, patient adopted chemotherapy before and after development of metastasis as well as for the case of resistance against the first line treatment.