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Fluorescence lifetime imaging microscopy of chemotherapy-induced apoptosis resistance in a syngenic mouse tumor model

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During cancer therapy with DNA-damaging drug-agents, the development of secondary resistance to apoptosis can be observed. In the search for novel therapeutic approaches that can be used in these cases, we monitored chemotherapy-induced apoptosis resistance in a syngenic mouse tumor model. For this, syngenic murine colorectal carcinoma cells, which stably expressed a FRET-based caspase-3 activity sensor, were introduced into animals to induce peritoneal carcinomatosis or disseminated hepatic metastases. This syngenic system allowed *in vitro*, *in vivo* and *ex vivo* analysis of chemotherapy induced apoptosis induction by optically monitoring the caspase-3 sensor state in the tumor cells. Tumor tissue analysis of 5-FU treated mice showed the selection of 5-FU-induced apoptosis resistant tumor cells. These and chemo-naive fluorescent tumor cells could be re-isolated from treated and untreated mice and propagated in cell culture. Re-exposure to 5-FU and second line treatment modalities in this ex-vivo setting showed that 5-FU induced apoptosis resistance could be alleviated by imatinib mesylate (Gleevec). We thus show that syngenic mouse systems that stably express a FRET-based caspase-3 sensor can be employed to analyse the therapeutic efficiency of apoptosis inducing chemotherapy.

DNA-damaging compounds such as 5-fluorouracil (FU) induce apoptosis by affecting DNA synthesis and repair mechanisms.[1] The response to DNA damage is either repair or cell death, and therefore results clinically in chemo-resistance or tumor chemosensitivity.[2] The capacity of a cancer cell to repair DNA essentially determines the resistance to chemotherapeutic drugs that induce DNA damage.[3] Several signaling pathways have been shown to arrest the cell cycle following DNA damage to allow time for DNA repair. When repair remains incomplete, *i.e.*, in cases of excessive DNA damage, cells will still undergo apoptosis or go into senescence.[4] Chemoresistance may therefore be defined as failure of tumor cells to undergo apoptosis in the presence of DNA-damaging compounds. Tumors may be either intrinsically resistant to chemotherapy prior to treatment or acquire resistance during treatment after initial sensitivity.[2,3]

The possibility to investigate cancer experimentally in rodent models has provided insights into substeps and mechanisms of disease propagation and guided efforts towards novel cancer therapies. We have previously established a syngenic murine model for colorectal cancer using EGFP as marker protein to determine the tumor load within Balb/c mice.[5] The murine C-26 cells used in this model have been described to undergo apoptosis under chemotherapy which implies that they are not primarily resistant to cytotoxic treatment.[6] Similar syngenic tumor models have also been developed for squamous cell lung cancer, protastatic cancer and malignant melanoma.[7–9] While these models allow evaluation of early tumor development and chemotherapy treatment efficiency by *in vivo* detection of cells,[5] they do not provide any information on the molecular mechanisms affected by chemotherapy. To better understand the molecular background of tumor development such as chemotherapy induced apoptosis resistance, it is necessary to observe key molecular mechanisms which are involved in apoptosis induction *in vivo*. This could allow the development of novel therapies by facilitating direct monitoring of the influence of chemotherapeutic drugs on molecular reactions that determine the fate of the cell.

Optical imaging of specific molecular targets and pathways in living cells has recently become possible through continued developments in microscopic imaging technology, and more importantly, the availability of genetically encoded fluorescent biosensors.[10,11] These fluorescent reporters have revolutionised live-cell imaging of biochemical processes in a variety of cells from different organisms[12–14] since these proteins can directly be used to study protein reactions in living cells by fluorescence resonance energy transfer (FRET).[15,16] Accordingly, FRET between tandems of fluorescent protein

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