Title: {Development of a new cell death assay for monitoring effective T cell immunotherapy of cancer.}

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Abstract:
{Immunotherapy is considered an important strategic approach in the treatment of cancer. However, the development of cancer vaccines is hindered by the lack of relevant biological models due to the complexity of the immune response where simultaneous multiple readouts are required. Therefore, we have developed a new cell based assay that, due to multiple optical readouts, allows simple analysis of CD8+ T cell mediated cancer cell death. The optical readouts are based on bioluminescence of luciferase expressing tumor cells and the use of a new Near InfraRed Fluorescent (NIRF) cell death probe HQ5 that recognizes late apoptotic and necrotic cells that have lost membrane integrity.

In particular we used bioluminescent luciferase expressing MC38-luc colon carcinoma cells and bioluminescent MC38-OVA-luc cells that also express the ovalbumin (OVA) antigen. Cancer cells were incubated with activated CD8+ T-cells isolated from the spleen of OT-1 mice that are able to recognize and kill OVA expressing cancer cells. Cancer cells were incubated with increasing amounts of T-cells and analysis was performed at different time points. The addition of luciferin and the NIRF cell death probe HQ5 allowed the simultaneous monitoring of living/dead cells at different time points in a 24- and 96-well plate format. In contrast to non-OVA expressing MC38-luc cells, the bioluminescent signal from the ovalbumin expressing MC38-OVA-luc cells decreased with increasing numbers of T-cells, indicating that the tumor cells were killed by the T-cells. On the other hand, HQ5 fluorescence increased in these samples, indicating that the amount of fluorescence can be used as a marker for cell death. These results indicate that the NIRF dye HQ5 can be used for the quantification of T-cell induced tumor cell death in vitro. We are now in the process of performing these studies in vivo.}

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