

## HEAT SHOCK INCREASES ANTIGEN-SPECIFIC T CELL ACTIVATION USING PRIMARY SARCOMA CELLS AND CELL LINES TRANSFECTED WITH MVA-HTYR

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### Background

Sarcomas are malignant mesenchymal neoplasms that are characterized by a wide spectrum of histopathologic features. Because of the unpredictable clinical behaviour and the lack of objective surrogate markers for its evaluation, progress in the management of these tumors has been minimal.

In recent years different approaches for the development of an anti tumor vaccine based on the activation of antigen-specific T cells have been very promising. Crucial for monitoring the potential of each vaccine of inducing a specific T cell activation is the knowledge of at least one antigenic determinant within the vaccine. We transfected sarcoma cells with the tyrosinase gene. This offers the possibility of monitoring tyrosinase specific immune response independent of sarcoma specific antigens. Here we studied the potential of heat shock to augment the activation of antigen specific T cells by tyrosinase-transfected sarcoma cells.

### Materials and Methods

Primary sarcoma cells of patients and sarcoma cell lines RD-ES and MG63, all endogenously negative for tyrosinase, were infected with MVA (modified vaccinia virus Ankara) containing the complete sequence of human tyrosinase using different MOI (multiplicity of infection). Using MVA-expressing GFP (green fluorescence protein) the percentage of infected cells was assessed by flow cytometry and fluorescence microscopy. Tyrosinase expression was detected by western blot analysis. Tyrosinase transfected cells with and without additional heat shock treatment (41,8°C; 90 min) were coincubated with the HLA-A\*0201-restricted tyrosinase peptide tyr368-376-specific cytotoxic T-cell clone TyrF8. T cell activation was assessed by IFN- $\gamma$  ELISA and on a single cell basis by IFN- $\gamma$  ELISPOT. As control we used the tyrosinase peptide independent, HLA-A02-alloreactive, T cell clone JB4.

### Results

Viability of cells after infection with MVA was usually > 90%. Percentage of MVA-GFP-positive cells and tyrosinase expression after infection with MVA-hTyr was dependent on the MOI, with a maximum at MOI 10. Highest tyrosinase protein expression was detected 16 hours after infection with MVA-hTyr for one hour. HLA-0201 positive primary cells and cell lines (MG63) induced IFN- $\gamma$  production by tyrosinase-specific T cell clone TyrF8. HLA-0201 negative control cell line RD-ES did not lead to any T cell activation despite tyrosinase expression after MVA-hTyr infection. IFN- $\gamma$  synthesis by tyrosinase-specific T cell clone TyrF8 was increased by additional exposure of sarcoma cells to heat shock before MVA-hTyr infec-

tion. These results could be confirmed on a single cell level by ELISPOT analysis. Heat shock is a valuable tool to increase the immunogenic potential of antigenic peptides.