

# A Novel Strategy for the Identification by Phage Panning *in Vivo* of Ligands Specific for Cells in the Extra-vascular Space.

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Surface molecules exclusively expressed by cells of the bone marrow (BM) are candidate targets for therapeutic delivery to this tissue. To identify ligands specific for the BM we screened a series of random peptide phage displayed libraries *in vivo*. Several phage clones apparently BM-specific could be identified after 5 to 6 consecutive panning cycles. However, a consensus motif did not emerge and synthetic peptides were unable to inhibit the bone marrow homing of the corresponding phages. Furthermore, phages bearing irrelevant peptides or no peptide showed a strong "natural" affinity to the BM endothelium (BME) which was abrogated by polyanions. These results indicate that the BME is part of the reticulo-endothelial system (RES). They also suggest that phage trapping by this endothelium is mediated by scavenger receptors (SR). To circumvent interference by SR, poly-inosinic acid (Poly-I) was administered prior to phage panning *in vivo*. This led to the identification of phage clones bearing a consensus motif that confers binding specificity for a sub-population of hemopoietic marrow cells. Thus, SR inhibition, by avoiding phage trapping by the endothelium, seems to allow phage particles to extra-vasate and reach pericythelial cells. Accordingly, this panning strategy *in vivo* may be useful for the identification of targeting motifs specific for cells located in the extra-vascular space of various tissues.

# STAPHYLOCOCCAL ENTEROTOXIN B- MEDIATED T CELL IMMUNE RESPONSE INDUCES APOPTOSIS IN BLADDER CANCER CELLS.

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Potent activators of T lymphocytes are toxins produced by *Staphylococcus aureus*. The enormous potency of these molecules is reflected in several preclinical and clinical studies in cancer therapy. We initiated this study to evaluate SEB-activity as a new approach in therapy for bladder cancer. Since the Fas-system is a major mediator of apoptotic cell death we first examined if SEB (staphylococcal enterotoxin B) can induce Fas-ligand expression on peripheral blood mononuclear cells (PBMC). Second we evaluated cytokine secretion by PBMC after SEB treatment and finally investigated if SEB-activated PBMC are able to induce apoptosis in human transitional cell carcinoma cells (TCC) *in vitro*. We demonstrate by flowcytometric analysis pronounced time-dependant induction of Fas-ligand on PBMC by SEB. Further, ELISA assays show a massive, dose dependant secretion of cytokines IL-2, IFN-gamma and TNF-alpha released from the SEB-stimulated PBMC. In co-culture experiments we demonstrate that SEB-activated peripheral blood mononuclear cells kill TCC cells by Fas-ligand. TCC cells treated with culture supernatant of SEB-treated PBMC demonstrated a minimal response only. Application of PBMC at the time of maximum Fas-ligand expression and most pronounced cytokine secretion lead to a massive TCC cell death. These results suggest that further evaluation of SEB as a potential candidate for immunotherapy of bladder cancer is warranted.

# Inactivation of the *INK4* gene cluster in mantle cell lymphomas - standardization and initial analysis of differential methylation patterns of cell cycle regulators

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Mantle cell lymphoma (MCL) is characterized by the chromosomal translocation t(11;14)(q13;q32), resulting in the overexpression of cyclin D1. However, additional genetic alterations (like deletions of the *INK4A* gene cluster) are detectable in the vast majority of cases. The hypermethylation of CpG islands of the promoter region represents an alternative mechanism of gene inactivation. So far, the hierarchy of *p15<sup>INK4B</sup>*, *p16<sup>INK4A</sup>*, and *p16<sup>ARF</sup>* promoter methylations as well as the interaction of different methylation sites within a distinct promoter region is poorly understood.

Based on previously published methods (Herman et al., PNAS 1996) we have established a comprehensive method to exactly determine the promoter methylation pattern of the *INK4* gene cluster. In an initial series of 5 hematological cell lines, the methylation pattern of *p15<sup>INK4B</sup>*, *p16<sup>INK4A</sup>*, and *p16<sup>ARF</sup>* could be confirmed and characterized in more detail in all samples analysed.

Currently, we have initiated a complete methylation analysis of 52 MCL which had been previously characterized for other *INK4A* gene cluster (genomic deletion) and *p53* (mutations, expression) alterations to more accurately define the differential impact of *p15<sup>INK4B</sup>*, *p16<sup>INK4A</sup>*, and *p16<sup>ARF</sup>* methylation patterns in mantle cell lymphomas.

# IN-VITRO TNF-α EFFECTS ON HEMATOPOIETIC CELL LINES

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TNF-α *in vitro*, either suppresses the growth of some myeloid cell lines or stimulates the growth of some lymphoid cells. Based on possibility of TNF-α to make diverse effects on hematopoietic cells, evaluation of cell membrane surface antigen expression before and after TNF-α *in-vitro* treatment, was important for understanding its effects following cell death or cell activation process. In this work we analyzed antigen expression on K-562 (erythroleukemic), Raji (malignant B cell line) and PC (MDS syndrome) cell lines before and after TNF-α treatment. Cell lines were cultured with or without TNF-α, 500 and 1000 pg/ml of culture medium and after 18h cell membrane antigen expression and apoptotic and necrotic form of cell death, were analyzed by flow cytometer (Becton-Dickinson) on gated cell population. For discrimination of apoptotic from necrotic form of cell death process cells were pre-labeled with annexin V and propidium iodide. Results shows that TNF-α in a dose-dependent manner significantly decrease of cell membrane expression on hematopoietic cell lines for evaluated antigens, for more than 2 folds, calculated from basal values expression. In addition, decrease of antigen expression did not correlated with apoptotic form of cell death process in all examined cell lines. Decrease of some antigen expression, which partly TNF receptor superfamily members, after TNF-α treatment suggested their ineffectively for induction of apoptotic process but more for activation of the necrotic process.