

## PROTEASOME INHIBITORS MODULATE CHEMOKINE PRODUCTION IN LUNG EPITHELIAL AND MYELOID CELLS

Inhibition of proteasome function results in delayed tumor growth and sensitisation of tumor cells to apoptosis. Therefore these inhibitors are assumed to be promising drug candidates for the therapy of various cancer types, such as lung cancer which is associated with a high mortality rate.

The present study demonstrates that the proteasome inhibitors ALLN, MG-132 and  $\beta$ -lactone are capable of suppressing the proliferation of the lung epithelial cell line A-549 in a dose-dependent manner. Inhibition of proteasome function was found to result in an accumulation of the cell cycle regulatory protein cyclin B and in a G2/M cell cycle arrest. These effects were not due to an inhibition of lysosomal cysteine proteinases, since the cysteine protease inhibitor E-64d had no effect.

Moreover, inhibition of proteasome activity induced a significantly increased IL-8 secretion by different human lung epithelial cell lines, primary lung cells as well as by myeloid cell lines. The IL-8 induction in response to proteasome inhibition was detectable at protein and mRNA levels but varied between the different cell types. In the levels of induced IL-8 production there are cell-type-dependent differences. Further studies revealed that the inhibitor induced elevated IL-8 production based on both, an increased transcription rate of the IL-8 gene plus a prolongation of the IL-8 mRNA half-life.

The promoter region of the IL-8 gene is known to contain binding sites for the transcription factors NF- $\kappa$ B, AP-1 and NF-IL-6. There are no hints in literature for a crucial role of NF-IL-6 in regulation of IL-8 expression in A-549 cells. The NF- $\kappa$ B dependent regulation of the IL-8 gene transcription seemed to be blocked in the presence of proteasome inhibitors, since both the p50 and p65 subunit were found to be down-regulated. On the other hand the experimental data shown here provide evidence that the proteasome inhibitor induced IL-8 gene transcription is mainly triggered by activation of AP-1 associated with an increased DNA binding activity of c-Jun proteins.

In order to prove the functional relevance of the secreted IL-8 its chemotactic activity was assessed by using the neutrophil chemotaxis assay. These experiments revealed that IL-8 in supernatants of proteasome inhibitor-treated cells is indeed capable of attracting isolated neutrophils *in vitro*.

In conclusion, the proliferation suppressing effect of proteasome inhibitors, their capability to regulate cell cycle progression and the effects on the expression of IL-8 demonstrated here are basis for future development of proteasome inhibitors as drug candidates for lung cancer therapy.