

SUMMARY

Background: Granzyme B (GrB) is a key proapoptotic secretory protease of CTLs and NK cells. Its specific proapoptotic effects in cancer cells can be blocked by increased expression of serpinB9. *SerpinB9* gene expression can be transcriptionally upregulated by some interleukins and by the oestrogen activated oestrogen receptor- α (ER α) in cells which express ER α protein. The aims of my thesis were to evaluate the expression of SB9 and to examine its inhibitory activity against exogenous active GrB in non-small cell lung carcinoma (NSCLC) cell lines and tissues. To analyse the expression status of GrB mRNA in NSCLC cell lines and tissues. To investigate the role of estradiol-17 β (E2), selected ILs and DNA methylation in regulation of SB9 expression in NSCLC cells.

The apoptosome apparatus is a cell death signalling platform activates the initiator procaspase-9. Activation of the apoptosome apparatus is often impaired in various types of cancer but the molecular basis of its suppression is still unknown. APIP and UACA/nucling belong to the endogenous regulators of apoptosome apparatus. The aim of my thesis was to investigate whether DNA methylation is involved in the transcriptional regulation of expression of *APIP* and *UACA* genes in NSCLC cell lines.

Methods: Following methods were used in this thesis: isolation and quantification of total RNA, real-time RT-PCR analysis, Western blot analysis, enzyme analyses, cell culture techniques and immunocytochemistry of ER α .

Results and conclusion: NSCLC cells express both SB9 mRNA and protein and there is a subset of NSCLC cell lines and tumours with upregulated SB9 mRNA and protein expression. Expressed SB9 protein is functional as it can interact with the active GrB via forming an irreversible complex GrB•SB9. SB9 mRNA expression was particularly upregulated in the less-differentiated adenocarcinomas from surgically treated patients. E2 and interleukins -1 β , -6, and -18 do not markedly up-regulate the SB9 expression in NSCLC cells. On the contrary, DNA methylation can profoundly down-regulate the expression of SB9 in a subset of NSCLC tumours. This suggests that DNA demethylating drugs might desensitize NSCLC cells against the granzyme B-induced apoptosis through a strong induction of SB9 expression. NSCLC cells and tumours which are high expressors of SB9 may be protected, via the constitutively or inducibly high levels of SB9, against the GrB-mediated apoptosis during the immune attack executed by cytotoxic lymphocytes and NK cells. DNA methylation is not significantly involved in the regulation of transcriptional expression of *APIP* and *UACA* genes in NSCLC cells.