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Dissertations-Kurzfassung

Methylation and gene expression of c-FLIP and caspase-8 in thymomas and thymic carcinomas and their relevance for the pathogenesis of thymic tumors

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Background: While anti-apoptotic transcriptomic and proteomic signatures have been known to be associated with thymic epithelial tumors as compared to the normal thymus, the underlying mechanisms have remained enigmatic. Whether methylation profiles of apoptosis-related genes are associated with Myasthenia gravis (MG) has been unknown as well.
Therefore, the current thesis had the following aims:
i.)To determine the methylation status of the promoters and expression of c-FLIP and caspase-8 on the RNA and protein levels across the histological spectrum of thymomas and thymic carcinomas compared to normal thymus using whole tissue extracts. ii.) To determine c-FLIP and caspase-8 expression and the methylation status of their promoters in ex vivo isolated thymocytes and in primary, short-term cultured epithelial cell lines derived from the respective tumors and thymuses. iii.)To determine the functional impact of the demethylating agent, 5-azacytiidine on the expression levels of c-FLIP and caspase-8 in the aforementioned primary, short-term epithelial cell cultures and the bona fide TC cell line 1899c. iv.) To determine caspase-8 as a possible new diagnostic marker in immunohistochemical analyses.
Available material: 82 snap frozen tissue samples of various thymoma histotypes (n=67), thymic squamous cell carcinomas (TSCC; n=15) and normal thymuses (n=11); 29 formalin-fixed, paraffin embedded cases; isolated thymocytes from lymphocyte- rich thymomas (n=9); the thymic carcinoma cell line, 1889c and several control cell lines. Methods applied: Methylation-specific PCR (MSP), qRT-PCR, immunohistochemistry, Western Blots and functional in vitro studies (demethylation of 1889c cells followed by methylation-specific PCR to quantify c-FLIP and caspase-8 expression.
The results can be summarized as follows: i.) The c-FLIP promoter was hypomethylated and c-FLIP protein expression was increased in thymic epithelial tumors as compared to non-neoplastic thymus. ii) The caspase-8 promoter was hypermethylated and expression of caspase-8 protein was lower in aggressive thymic cancers (B2, B3 thymomas, TSCC) than in indolent thymomas
(A, AB thymomas) and non-neoplastic thymus. iii) Treating 1889c thymic carcinoma cells with a demethylating agent did not increase c-FLIP protein expression (i.e. did not increase this anti-apoptotic feature of thymic tumors) but resulted in increased levels of cleaved caspase-8 (i.e. increased a pro-apoptotic feature); iv) There was no correlation between c-FLIP/caspase-8 promoter methylation and gene expression on the one hand and the occurrence of Myasthenia gravis (MG) on the other hand.
Conclusion: The current findings suggest that epigenetic mechanisms, DNA-methylation in particular, might contribute significantly to the oncogenesis of thymic epithelial tumors and might be promising therapeutic candidate targets. The low number of myasthenic versus non-myasthenic thymomas in the present study precludes conclusions as to whether or not the methylation status of the c-FLIP and caspase-8 genes plays a role in the pathogenesis of MG.