Summary

DNA methylation changes as potential biomarkers in the treatment of bladder cancer

Introdution
Bladder Cancer (BC) is the second most common malignancy of the urinary tract. BC has two categories. Approximately 75 % of patients with BC present with disease confined to the mucosa or submucosa (non muscle invasive bladder tumor – NMIBC). Carcinoma in situ (CIS) is a very specific subgroup of NMIBC, because it is not a papillary lesion but a flat tumor, which is why CIS can be missed in cystoscopy. CIS is always a high grade tumor. Without any treatment, approximately 54% of patients with CIS progressed to muscle-invasive or metastatic disease. The second category covers patients with muscle-invasive BC (MIBC). These patients have a higher prevalence of progression rates and higher cancer-specific mortality. Patients with NMIBC are indicated to transurethral tumor resection (TUR) alone or with adjuvant treatment (intravesical chemotherapy or intravesical Bacillus Calmette-Guérin - *BCG* immunotherapy). BCG is an attenuated mycobacterium developed as a vaccine for tuberculosis that has demonstrated antitumor activity in BC intravesical instillation, and significantly reduces the progression risk of high grade papillary lesion and CIS. The therapy of MIBC is radical cystectomy – hard mutilating surgery with urinary diversion. This procedure has significant impact on the quality of the patient's life. Patients with NMIBC high grade tumor and CIS are the focus of our interest. It is a borderline subgroup, where radical and non-radical treatments are both possible. Non-radical therapy means complete tumor resection with adjuvant intravesical immunotherapy (BCG vaccine). Radical therapy means radical cystectomy. At present we have no markers which would be helpful in deciding optimal therapy. Weighing the risk of failure of non-radical treatment against overtreatment by radical therapy constantly presents a dilemma. The etiology of BC is multifactorial, driven by the multistep accumulation of environmental, genetic and epigenetic factors. Our research was focused on differences methylation status in blader cencer tissue like the most known epigenetic alteration. A new model for the mechanism of carcinogenesis has been proposed in which hypermethylation of unmethylated cytosine-phosphate-guanine (CpG) islands in the promoter regions of tumour suppressor genes (TSG) in normal cells silence these genes and this leads to the cells becoming cancerous.

Aims
Genetic and epigenetic alterations play an important role in urothelial cancer pathogenesis. Deeper understanding of these processes could help us achieve better diagnosis and management of this life-threatening disease. The aim of this research was to evaluate the typical methylation status differences of selected tumor suppressor genes in malign tissues. Then these methylation status differences are used for predicting BCG response in patients with high NMIBC and CIS. Patients who underwent BCG instilation in our department were included in this research.

Methods
We evaluated retrospectively data 82 patients with high grade NMIBC and CIS who had undergone BCG instillation therapy. Patients who have met inclusion criteria; methylation statu of their urothelial malign tissues were analyzed. Tumor tissues were stored in pathological department. We used the MS-MLPA (Methylation-Specific Multiplex Ligation-Dependent Probe Amplification probe sets ME001 and ME004). The control group covered 13 specimens of normal urotel (bladder tissue). Then we compared epigenetic methylation status in BCG-responsive and BCG-failure groups.
Results

Newly identified methylations in high grade NMIBC were found in MUS81a, NTRK1 and PCCA. The methylation status of CDKN2B ($p=0.00312^{**}$) and MUS81a ($p=0.0191^{*}$) is associated with clinical outcomes of BCG instillation therapy response. CDKN2B and MUS81a unmethylation was found in BCG failure patients.

Conclusion

The results advocate the importance of epigenetic mechanism in carcinogenesis. The methylation status differences of selected tumor suppressor genes (TSGs) has the potential for predicting BCG response in patients with NMIBC high grade tumours. Tumour suppressor genes such as CDKN2b, MUS81a are very promising for future research.