RASSF1A and PRSS21 as Future Diagnostic Biomarkers for Testicular Germ Cell Tumors

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Testicular germ cell tumors (TGCTs) are the most common malignancies in young men with an overall increasing incidence. Therefore, it is crucial to identify biomarkers for early detection and diagnosis. TGCTs are divided into seminomas (SE) and nonseminomas (NS) that both arise from germ cell neoplasia *in situ* (GCNIS). *RASSF1A* is a tumor suppressor gene that influences tumor initiation and development. *PRSS21* is expressed in normal testes and it is hypothesized to be a tumor suppressor gene. In contrast to *RASSF1A* and *PRSS21* gene expression status and DNA methylation pattern, its protein expression is poorly investigated. Therefore, we performed a comprehensive in silico bioinformatics analysis at the DNA methylation level and mRNA level and compared it with data of protein expression in TGCTs. *RASSF1A* and *PRSS21* protein expression was analyzed in 108 TGCT samples by immunohistochemistry in healthy testicular seminiferous tubule tissue (HT), GCNIS, TGCTs, SE, and all NS components (embryonal carcinoma, yolk sac, choriocarcinoma, teratoma). The immunoreactivity score (IRS) was calculated with a cut-off value of four for clinical diagnostic positivity. UALCAN was used for the analysis of promoter methylation levels in SE and NS. The highest *RASSF1A* and *PRSS21* protein expression were in GCNIS, while in HT and TGCTs was significantly lower. In HT and TGCTs, *RASSF1A* and *PRSS21* showed inverse protein expression to their mRNA levels. In SE, *RASSF1A* expression on mRNA level was significantly higher than in NS, while differences of *PRSS21* in mRNA expression were not found. *RASSF1A* and *PRSS21* show general hypomethylation in SE and hypermethylation in NS, although no differences in protein expression were detected. Regarding NS components, *PRSS21* has shown increased protein expression in choriocarcinoma. *PRSS21* and *RASSF1A* discriminate GCNIS from HT and TGCT, as well as SE from NS, showing significant potential as future TGCTs biomarkers on more than one molecular level.