Abstract

Testicular germinal tumors (TGCT) are relatively rare solid tumors in adults. Even so, they affect more than 700 men a year in the Czech Republic, mostly young patients aged 18-45 years. A large number of patients are curable by a combination of surgery and chemotherapy, yet about 50 men a year in the Czech Republic succumb to this tumor, in the vast majority of cases due to the development of resistance to chemotherapy containing cisplatin. The rare occurrence and high curability are probably the cause of infrequent molecular and clinical studies carried out in these tumors, and our understanding of the biological processes leading to primary tumor development and the development of cisplatin resistance (CDDP) is still limited. At present, no specific molecular markers that could be used as prognostic or predictive factors and improve patient stratification or treatment tailoring are available in TGCT management. In this work, we studied the molecular-genetic background of TGCT development and CDDP resistance at several levels. To comprehensively study the development of cisplatin resistance, we prepared and analyzed CDDP-exposed TGCT cell lines. Long-term exposure to CDDP increased resistance 10-fold in the NCCIT cell line, while no significant resistance was achieved with Tera-2. The development of CDDP resistance was accompanied by a transient decrease in proliferation and changes in the cell cycle - an increase in G1 and a decrease in S-fraction in the cell population. CDDP-resistant NCCIT cells showed more aquired mutations (a total of 21, including 3 in the *ATRX* gene), as well as significant changes in gene expression (eg. *PAX5* gene). Analysis of copy number variations (CNV) showed large losses in copy number of multiple genes and several duplications in CDDP-resistant cells. We further investigated the significance of Wilms‘ Tumor gene 1 (*WT1*) in TGCT as it is a transcription factor necessary for testicular development and function and its changes have been associated with various other malignancies, so we assumed its potential role in the pathogenesis of TGCT. Quantification of the expression of total *WT1* and its major isoforms by qPCR in 105 TGN patients (114 tumor samples and 100 controls) showed a significant decrease in *WT1* expression with a significant shift to the isoforms lacking exon 5, which, together with a relatively high incidence of *WT1* somatic mutations, identified *WT1* as a novel potential factor involved in the pathogenesis of TGCT, probably as a tumor suppressor gene. In a cohort of 210 patients with TGCT, the *BRAF* V600E mutation was detected by qPCR, and this was identified in only 1 % of patients, suggesting its no significance in TGCT development. Expression of the p53 protein was assessed by immunohistochemistry, demonstrating a

significant decrease of expression in metastases compared to the original tumors. These results may suggest a role for p53 in the metastatic spread in TGCT. Our work further includes sequencing data from 31 patient primary tumor samples and paired controls. There were significant aberrations of multiple genes found, with the highest number of mutations being observed in patients who developed CDDP resistance. Interestingly, in 2 patients a mutation in the *ATRX* gene was detected. Aberrations were also found in selected genes during amplicon sequencing, which may be important in the biology of TGCT – e.g. *ATM, WT1* and *RAS* genes. Our research highlights molecular aberrations related to the development of cisplatin resistance or the pathogenesis of TGCT, some of which may be implicated in the clinical practice in the future.