10 Summary

In the present study, a proteome-based approach was chosen to identify and characterise proteins that are associated with the molecular mechanisms underlying the prognostic significance of a combined loss of heterozygosity (LOH) on chromosome arms 1p and 19q. Therefore, several experiments were performed and following results obtained:

- Determination of 1p/19q LOH status in 16 glioma samples using microsatellite analysis detected combined loss of heterozygosity (LOH) on 1p and 19q in 3 glioma samples.
- Protein expression profiles of 9 WHO grade II oligoastrocytomas, including 5 tumors without and 4 tumors with loss of heterozygosity on both chromosomal arms revealed 72 regulated protein spots by applying the following criteria: \( p \leq 0.05 \) and fold change \( \leq -0.55 \geq 1.8 \).
- By means of MALDI-MS 43 protein spots (60%), representing 22 non-redundant proteins, were identified. From these candidates, only the genes for alpha-enolase (\( ENO1; \) 1p36.3-p36.2) and peroxiredoxin 1 (\( PRDX1; \) 1p34.1) map to chromosome 1p.
- Western blotting comprising 19 glioma samples corroborated the DIGE/MALDI-MS results by demonstrating lower expression levels of annexin A1, glial fibrillary acidic protein, peroxiredoxin 1, vimentin and ezrin in 1p/19q-deleted tumors.
- Lower relative expression levels of annexin A1, glial fibrillary acidic protein, vimentin and ezrin in 1p/19q-deleted gliomas were confirmed by immunohistochemistry analysing an extended tumor panel (43 glioma samples). All four proteins revealed good accuracy for glioma subgroup stratification in the ROC analysis.
- In line with the protein expression data, real-time reverse transcription-PCR analyses revealed significantly lower mean transcript levels of \( VIM \) and \( ANXA1 \) in 1p/19q-deleted gliomas (Student’s t-test, \( p < 0.01 \) and \( p < 0.05 \), respectively).
- Furthermore, sequencing of sodium bisulfite-treated DNA of 33 tumor specimens revealed more frequent methylation of the 5’-CpG islands associated with the \( VIM \) and \( EZR \) genes in 1p/19q-deleted gliomas as compared to gliomas without these deletions.

To study the effects of the candidate proteins in respect to increased chemosensitivity of 1p/19q-deleted gliomas a cell culture model of overexpression and knock-down was established for 4 candidate proteins (annexin A1, intracellular chloride channel protein 1, peroxiredoxin 1 and ezrin) in two glioblastoma cell lines (A172 and T98G).
• The treatment of all generated cell lines with BCNU, temozolomide and vincristine proof that all analysed candidate proteins are involved in response to chemotherapy.

• Especially, in all cases the BCNU treatment of the cell lines with up- or down-regulated candidate genes resulted in a chemoresistant or chemosensitive phenotype, respectively.

• A treatment with temozolomide and vincristine caused an increased mortality (chemosensitivity) in all cell lines overexpressing the candidate genes. The opposite effects (reduced mortality, chemoresistance) in the knock-down cell lines confirmed the results.

The chosen approach of differential proteome analysis and subsequent investigation of candidate proteins in cell culture led to candidate proteins functional involved in chemosensitivity of 1p/19q deleted glioma. The experiments using four different candidate proteins and three chemotherapeutic drugs with different biological functions and cytotoxic effects, respectively, suggest the existence of different mechanisms mediating chemoresistance in gliomas without 1p/19q deletion. The results of the proteins (intracellular chloride channel protein 1 and peroxiredoxin 1) involved in detoxification of cells probably show that a deregulated antioxidative system plays a major role.