Supplementary results

Protein expression in functioning PanNETs other than insulinomas and non-functioning PanNETs

Detailed data on PanNET, other than insulinomas, patient’s age, sex, metastatic disease, disease stage and tumor grade and size are provided as Supplementary Table 6.

TMAs with tissue cores of 12 gastrinomas, 11 glucagonomas, 10 vipomas and 59 non-functioning (NF) pancreatic tumors were analyzed for protein expression (Supplementary Table 7).

Similar to insulinomas, no EGFR expression was detected. While in 22% of insulinomas positive nuclear staining for p-AKT was observed, this was hardly detected in other PanNETs. p-ERK expression was observed in all tumor subtypes in approximately 25% of cases. IGF2, IGF1R and INSR were expressed in 40-100% of all PanNET subtypes, except for membranous IGF1R and cytoplasmic INSR expression in glucagonomas (both 33%). p-S6K and p-4EBP1 immunostaining was observed in a relatively low percentage of different PanNET subtypes. Thus, also PanNETs other than insulinomas show high expression levels of IGF2, IGF1R and INSR and low levels of mTOR pathway proteins.

A correlation between cytoplasmic IGF1R and cytoplasmic INSR (p=0.033) was identified when analyzing the combined data obtained for gastrinomas, glucagonomas and vipomas. In non-functioning PanNETs, a correlation of IGF2 with cytoplasmic IGF1R (p=0.001) was found, similar to insulinomas, as well as with cytoplasmic p-ERK (p=0.016). Cytoplasmic IGF1R also correlates with cytoplasmic p-ERK (p=0.043), and cytoplasmic INSR with nuclear p-ERK (p=0.001).

Correlation of protein expression with tumor grade, size and metastatic disease in functioning PanNETs other than insulinomas and non-functioning PanNETs
After comparing the mean relative protein expression levels in the combined group of gastrinomas, glucagonomas and vipomas no differences between grade 1 and grade 2/3 were found (Supplementary Table 8). In non-functioning PanNETs, grade 2/3 tumors showed a significantly lower IGF1R and IGF2 expression level than grade 1 tumors \((p=0.044\) and \(p=0.052\), respectively). No statistically significant differences were found between non-metastatic and metastatic non-functioning PanNETs, but when comparing tumors \(<2\text{cm}\) and \(\geq2\text{cm}\), nuclear p-ERK expression was significantly higher in the latter subgroup \((p=0.020)\). Due to inequality no statistical analysis was performed in the group of gastrinomas, glucagonomas and vipomas with respect to tumor size and metastatic disease.

**No correlation of protein expression with survival in PanNETs other than insulinomas and non-functioning PanNETs**

Kaplan Meier curves revealed no correlations between 10 years disease-free or overall survival rates and protein expression levels, both in the combined group of gastrinomas, glucagonomas and vipomas as well as in non-functioning PanNETs.