mRNA expression of mTOR signaling components and *MGMT* promoter methylation status as potential predictive biomarkers in pancreatic neuroendocrine neoplasms

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INTRODUCTION

Medical therapeutic options for the treatment of well-differentiated pancreatic neuroendocrine neoplasms (pNENs) include the mechanistic target of rapamycin (mTOR) pathway inhibitor everolimus[1,2] and the alkylating cytotoxic agent temozolomide[3] (in combination with 5-Fluorouracil). Criteria to assess prognosis in pNEN patients are still limited, as biomarkers to predict the therapeutic response are still unknown. Several studies could show aberrant mTOR signaling pathway activity at different levels of the signaling cascade in pNENs. Moreover, the response to temozolomide correlates with the methylation-status of the promoter region of the O6-methylguanine-DNA methyltransferase (MGMT) gene in glioblastomas.

OBJECTIVES

We aimed to analyze the mRNA expression of different mTOR pathway components and evaluated the *MGMT* methylation status in patients with well-differentiated pNENs in order to identify potential prognostic and predictive biomarkers.

MATERIALS & METHODS

Real-time quantitative TaqMan reverse transcriptase-polymerase chain reaction was used to analyze mRNA expression[4] of eleven mTOR pathway genes (*IGF-1*, *IGFBP-3*, *PIK3CA*, *AKT-1*, *MTOR*, *MLST8*, *DEPTOR*, *RAPTOR*, *RICTOR*, *4EBP1* und *VEGF-A*), in tumor tissue of 75 patients with well-differentiated pNENs in comparison to normal pancreatic tissue. Relative gene expression was determined according to the 40-ΔCT method using *CALM2* as housekeeping gene. Furthermore, we used bisulfite conversion followed by pyrosequencing to determine the methylation status of the *MGMT* promoter in tumor tissue of 76 pNEN patients.

RESULTS

Characteristics	Number of patients in "mTOR collective" (n = 75)	% in "mTOR collective"	Number of patients in "MGMT collective" (n = 76)	% in "MGMT collective"
Sex				
Male	46	61.3	45	59.2
Female	29	38.7	31	40.8
Histopathology				
G1	24	32.0	24	31.6
G2	51	68.0	52	68.4
Hemangioinvasion	28	37.7	28	36.8
Lymphangioinvasion	12	16.0	12	15.8
Analysed tissue				
Pancreas	65	86.7	65	85.5
Liver	7	9.3	7	9.2
Lymph nodes	2	2.7	3	3.9
Soft-tissue metastasis	1	1.3	1	1.3
Initial UICC staging				
UICC I	7	9.3	7	9.2
UICC II	19	25.3	20	26.3
UICC III	13	17.3	12	15.8
UICC IV	36	48.0	37	48.7
Functional activity				
Yes	21	28.0	22	28.9
No	54	72.0	54	71.1

Table 1: Patient characteristics for the „mTOR" and „MGMT" collectives

Increased mRNA expression of mTOR signaling cascade components in pNEN

Comparing the mRNA expression of the eleven aforementioned mTOR-associated genes, we detected statistically significant different expression levels for *4EBP-1* (p = 0.001), *DEPTOR* (p = 0.005), *IGF-1* (p = 0.018), *IGFBP-3* (p < 0.001), *MLST8* (p = 0.009) and *RAPTOR* (p = 0.001) (Mann-Whitney U test) between pNEN samples of 75 patients and 11 normal pancreatic tissue controls (Fig. 1).

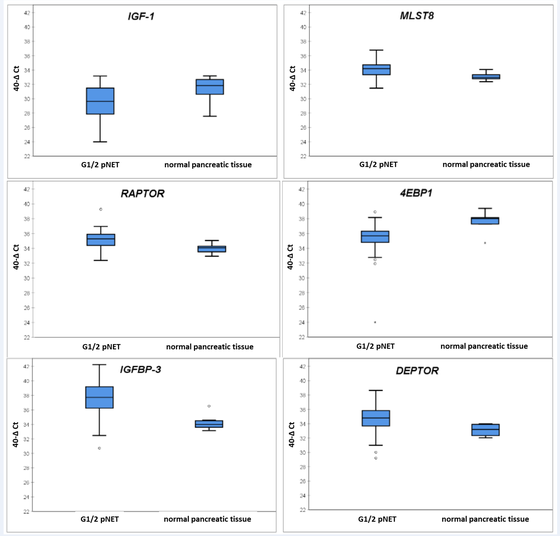

Figure 1: mRNA expression of the six significantly different expressed genes in pNETs in comparison to normal pancreatic tissue amongst the investigated mTOR pathway genes

RESULTS

High levels of RAPTOR mRNA expression correlate with overall survival

Clear association between mRNA expression and a clinical feature was only detected for *RAPTOR*. High *RAPTOR* mRNA expression was significantly correlated with better overall survival in unselected patients (Fig. 2).

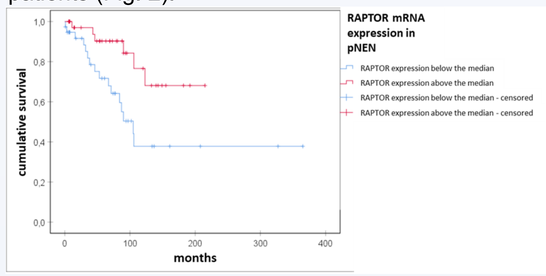

Figure 2: Kaplan-Meier curve for overall survival dependent on RAPTOR mRNA expression

No prediction to everolimus treatment response could be deduced by mRNA expression levels of mTOR pathway components in a subgroup of 21 patients.

Methylation status of the MGMT gene promoter region in pNENs

Hypermethylation of the *MGMT* promoter region occurred in 18.4 to 63.2 % of pNENs, dependent on different cut-off values used (Fig. 3). Neither MGMT protein expression, nor *MGMT* promoter methylation were associated with clinical characteristics (data not shown). The response to temozolomide was not correlated with *MGMT* promoter methylation status in a small subgroup of nine patients.

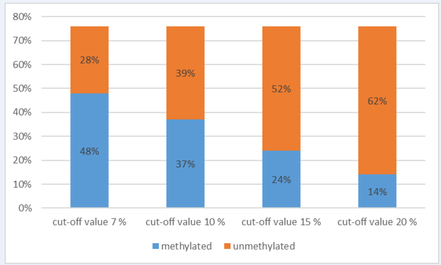

Figure 3: Methylation status of the MGMT promoter region using with cut-off values of 7 %, 10 %, 15 % or 20 %

CONCLUSIONS

Even though mRNA expression of different mTOR pathway components indicated an enhanced activity of genes involved in this signaling cascade in tumor tissue of pNEN patients, we could not identify a statistically significant parameter useful for prediction of everolimus treatment response. The prognostic and predictive value of *MGMT* promoter methylation status in pNENs is discussed controversially. We could not identify an association of *MGMT* status with clinical parameters in this study.

The predictive value of this study is limited due to the low number of patients who received treatment with everolimus or temozolomide. The advantage of our mRNA-based method is the possibility to evaluate multiple markers in one approach on one tissue sample. Further prospective studies analyzing the predictive value of the expression of mTOR pathway genes or the *MGMT* status are desirable.

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