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Research Article

Differential Gene Expression in Pancreatic Ductal Adenocarcinoma and Stromal Tissue: Prognostic and Therapeutic Implications

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ABSTRACT

Background: Pancreatic ductal adenocarcinoma is one of the most aggressive solid malignancies. The c-MET oncogene plays a crucial role in mediating local invasion, systemic dissemination and resistance in this cancer. The genetic makeup of surrounding stromal tissue has shown to be relevant for drug delivery in pancreatic cancer as exemplified by nab-paclitaxel binding to the stromal protein SPARK. In this study we investigated *c-MET, ENT1, EREG, GLUT1* and *RRM1* mRNA expression patterns in pancreatic ductal adenocarcinoma and stromal tissue in patients with clinical outcome.

Methods: FFPE tumor specimens from patients with resectable pancreatic cancer that underwent surgery and adjuvant chemotherapy with gemcitabine were evaluated. *C-MET, ENT1, EREG, GLUT1* and *RRM1* mRNA expression results could be obtained for 25, 25, 20, 25, 21 cases in tumor and 19, 21, 14, 20, 14 cases in stromal tissue as not all samples were sufficient in quality and quantity for microdissection and mRNA analysis. Specifically, designed primers and probes were used to detect mRNA *c-MET, ENT1, EREG, GLUT1* and *RRM1* expression levels by quantitative RT-PCR in reference to beta-actin.

Results: *C-MET, ENT1, EREG, GLUT1* and *RRM1* mRNA expression was significantly divergent between pancreatic stromal and tumor tissue ($p<0.0001$, $p<0.001$, $p<0.004$, $p<0.0001$, $p=0.48$). When statistically evaluated for the best cut-off, patients with high (>5.00) *c-MET* expression in the tumor tissue had a worse overall survival ($p<0.003$). *ENT1, EREG, GLUT1* and *RRM1* expression in the tumor tissue also influenced the overall survival ($p=0.398$, $p=0.106$, $p=0.050$, $p=0.199$). *C-MET* mRNA expression in stromal tissue did not correlate with outcome.

Conclusions: According to our data high *c-MET* expression is a negative prognostic indicator for pancreatic cancer. Further studies have to evaluate if *c-MET* expression may predict response to new *c-MET* inhibitors like cabozantinib. The role of *c-MET* expression in pancreatic stromal tissue needs further investigation.

Introduction

Pancreatic ductal adenocarcinoma (PDA) is one of the most aggressive solid malignancies and the fourth leading cause for cancer- related death in Europe and the U.S [1, 2]. Surgery as the only curative option is possible in only 10–20% of patients as PDA is often diagnosed in an advanced state, with an extensive local invasion or metastatic stage [3]. Despite the low-response rate and the modest overall survival benefit as well as fast development of resistance, gemcitabine, alone or in combination with other substances, is considered as standard chemotherapy for advanced pancreatic cancer [3]. The combination of 5-fluorouracil, leucovorin, irinotecan and oxaliplatin (FOLFIRINOX) extends life by only 4 months when compared to gemcitabine mono. However, this regime has severe side effects and, therefore, is only applicable for very few patients [4]. Consequently, it is essential to understand the influence of different gene expressions in ductal

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adenocarcinoma of the pancreas and stromal tissue on prognosis and therapy in order to enable new therapeutic approaches to improve the survival of patients with PDA. Several pathways and genes have been described as correlated with gemcitabine resistance in PDA. We therefore analyzed different gemcitabine resistance associated genes with respect to their expression on PDA cancer cells and the impact on overall-survival.

I c-MET

The receptor tyrosine kinase *c-MET* and its ligand *HGF* (hepatocyte growth factor) play an important role in embryogenesis and tissue regeneration [5-7]. Binding of *HGF* to its corresponding receptor *c-MET* leads to activation of intracellular signaling pathways including *MAPK/ERK*, *PI3K/AKT* and *FAK* [8]. In cancer, this confers multiple effects such as resistance to chemotherapy, induction of angiogenesis and promotion of metastasis [9]. With regards to pancreatic cancer, expression of *c-MET* has been associated with poor survival and phosphorylation of *c-MET* has been described in patients with early distant metastases even after complete surgical resection [10, 11]. Moreover, involvement of *c-MET* activation in resistance to gemcitabine therapy, tumor cell motility and secretion of angiogenic factors has been reported in pancreatic cancer [12-14].

II ENT-1

Gemcitabine is transported into the cell mostly by human equilibrative nucleoside transporter-1 (*hENT1*) [15]. Cells lacking *hENT1* are highly resistant to gemcitabine and pancreas cancer patients with *hENT1*-positive tumor tissue have significantly longer survival after gemcitabine chemotherapy than patients affected by tumors without detectable *hENT1* [16, 17].

III EREG

Epiregulin belongs to the epidermal growth factor (EGF) family of polyleptides. Zhu et al. compared in their study the expression and localization of epiregulin in the normal human pancreas and pancreatic ductal adenocarcinoma (PDA). It was shown that epiregulin may play a role in the pathobiology of PDA [18].

IV GLUT1

Glucose transporter type 1 (*GLUT-1*) is a glucose cell plasma membrane transporter. Increased glucose metabolism is a well-known characteristic of malignant cells [19, 20]. Enhanced glucose uptake in tumors is reflected by the overexpression of glucose transporter proteins. Glucose transporters, such as glucose protein type 1 (*GLUT-1*), mediate the first rate-limiting step in glucose transport and allow the energy-independent transfer of glucose down its concentration gradient [21]. Although *GLUT-1* is normally expressed in erythrocytes, endothelial cells, germinal centers of reactive lymph nodes, and several other additional sites, it is also expressed by pancreatic cancer cells [22, 23]. The metabolic consequences of increased glucose transporter remain unclear, but the overexpression seen in several human solid tumors has been associated with enhanced tumor aggressiveness and poor survival [24, 25].

V RRM1

RRM1 is the gene that encodes the regulatory subunit of ribonucleotide reductase and seems to be a key determinant of gemcitabine efficacy. *RRM1* is reported to influence cell survival, probably through interaction with the phosphatase and tensin homolog (*PTEN*), which is an inhibitor of cell proliferation, and suppresses cell migration and invasion by reducing the phosphorylation of focal adhesion kinase [26, 27]. Different studies have shown that in various cancers an overexpression of the *RRM1* gene is strongly associated with gemcitabine resistance [28, 29].
In this study we investigated *c-MET, ENT1, EREG, GLUT1* and *RRM1* mRNA expression patterns in pancreatic ductal adenocarcinoma and stromal tissue in patients compared to the clinical outcome.

Materials and Methods

Study Design and Patient Population

We conducted a retrospective analysis of data collected from a cohort of 26 patients with resectable pancreatic cancer that underwent surgery and adjuvant chemotherapy with gemcitabine, whose tumor tissue was submitted to Response Genetics Incorporated (Los Angeles, CA), a CLIA certified and CAP accredited laboratory, for comprehensive molecular testing. Formalin-fixed paraffin embedded (FFPE) tumor specimens were tested for mRNA expression levels of *C-MET, ENT1, EREG, GLUT1* and *RRM1*. Only patients whose specimens had sufficient tissue for analysis of at least one gene of interest (i.e. *C-MET, ENT1, EREG, GLUT1, RRM1*) as well as data regarding patient and tumor characteristics were included in this study. A total of 26 patients were included in the final analysis. Information regarding primary tumor location, patient age and gender, tumor grade and histology, were extracted from pathology reports submitted with the tissue specimens and recorded by two of the authors (C. P. B., P. S. P.). Tumor Tissue Preparation and Gene Expression AnalysisTumor tissue from study patients was obtained at the time of diagnosis prior to surgery and at the time of surgical resection. Hematoxylin and eosin (H&E) stained sections of all FFPE specimens were evaluated by a board certified pathologist for tumor content.

FFPE tissues were dissected. Ten-micrometer-thick slides were obtained from the identified areas with the highest tumor concentration and were mounted on uncoated glass slides. For histologic diagnosis, three sections representative of the beginning, middle, and end of the tissue were stained with H&E. Before microdissection, sections were de-paraffinized in xylene for 10 minutes, hydrated with 100%, 95%, and 70% ethanol, and then washed in H2O for 30 seconds. Following microdissection of tumor cells, the sections were stained with nuclear fast red (American Master Tech Scientific, Inc.) for 20 seconds and rinsed in water for 30 seconds. Samples were then dehydrated with 70%, 95%, and 100% ethanol for 30 seconds each, followed by xylene for 10 min. The slides were then completely air-dried. Laser capture microdissection (PALM Microlaser Technologies AG) was carried out in all tumor samples to ensure that only tumor cells were dissected [30]. The dissected particles of tissue were transferred to a reaction tube containing 400 mL of RNA buffer for lysis of tumor cells. After lysis of the tumor cells, RNA and DNA were isolated separately from the specimen. RNA isolation from paraffin-embedded samples was done

according to a proprietary procedure defined by Response Genetics, Inc. (US Patent #6248535). The RNA was then reverse-transcribed to cDNA as described previously [31]. DNA was either directly extracted or back extracted from the organic phase, both with an RGI patented method (US Patent #6248535).

Quantitation of gene mRNA expression levels of *C-MET, ENT1, EREG, GLUT1, RRM1* and an internal reference (*β-actin*) cDNA was done using a fluorescence-based real-time detection method [ABI PRISM 7900 Sequence detection System (TaqMan); Perkin-Elmer Applied Biosystem] as previously described [32]. Isolated RNA was reverse-transcribed to cDNA, followed by RT-PCR using specific primers and probes. The PCR reaction mixture consisted of 1,200 nmol/L of each primer, a 200 nmol/L probe, 0.4 U of AmpliTaq Gold Polymerase, 200 nmol/L of dATP, dCTP, dGTP, dTTP; 3.5 mmol/L MgCl2, and 1X TaqMan Buffer A containing a reference dye added to a final volume of 20 mL (all reagents from PE Applied Biosystems). Cycling conditions were 50ºC for 2 minutes, 95ºC for 10 minutes, followed by 46 cycles at 95ºC for 15 seconds and 60ºC for 1 minute. For each sample, parallel TaqMan PCR reactions were carried out for each gene of interest and the β-actin reference gene to normalize for input cDNA. Results were obtained as a ratio of the PCR fluorescent signals of each gene of interest relative to the reference gene, β-actin.

Statistical Analysis

Messenger RNA expression levels of *C-MET, ENT1, EREG, GLUT1, RRM1* were summarized and analyzed by Wilcoxon signed rank tests to detect differences within each tumor site. Pairwise differences between the expression of the five examined genes across tumor sites were then determined by Wilcoxon two-sample tests, with significance determined by Kruskal-Wallis testing. Bonferroni method was used to correct p value for multiple comparisons. All values were reported as medians and ranges, with a significance p-value cutoff ≤ 0.05. Analyses were performed using Statistical Analysis Software (SAS) version 9.3 (SAS Institute Inc. NC, USA).

Table 1: Patients' characteristics of the cohort (n=26) analyzed

Variable	Subtype	Number (n)	Percentage (%)
Gender	Male	12	46.2
	Female	14	53.8
Age (years)[1]		65.5 (45 – 83)	
Body mass index (BMI)[1]		22.49 (18.3 – 34.1)	
Tumor localization	Caput	16	61.5
	Corpus	2	7.7
	Cauda	8	30.8
Surgical procedure	pylorus-sparing pancreaticoduodenectomy according Traverso-Longmire	11	42.3
	Whipple	2	7.7
	distal pancreatectomy	6	23.1
	pancreatectomy	6	23.1
	other	1	3.8
Resected lymph nodes[1]		21 (10 – 66)	
T-category	pT1	0	0
	pT2	1	3.8
	pT3	25	96.2
N-category	pN0	7	26.9
	pN1	9	34.6
	pN2	10	38.5
R-category	R0	18	69.2
	R1	7	26.9
	Data missing	1	3.9
Follow-up (months)[1]		14.5 (3 – 45)	
Tumor recurrence[2]	Yes	19	73.1
	No	4	15.4
	Loss to follow-up	3	11.5
Localization of recurrence	Local	3	15.8
	Hepatic	4	21.1
	Disseminated	12	63.1
Time to recurrence (months)[1]		8 (1 -19)	

CTx: chemotherapy

[1]Median (Min.-Max.).

[2]missing information for three patients

[3]Data of patients who underwent surgery (n=26)

Results

I Demographic characteristics

There were 26 consecutive patients (12 males and 14 females) included within the current analysis who received diagnosis of PDAC between March 2008 and July 2011. The median age was 64.88 years (min: 45 years; max: 83 years) at the date of diagnosis. Tumor localization was pancreatic head in 16, pancreatic body/tail in 8 and pancreatic head/body in 2 patients. Presurgical stent implantation into the pancreatic duct was performed in 4 cases. All other patients (n=22) did never receive any stent during treatment. Median body-mass-index was 22.49 and ranged from 18.3 to 34.1. Recurrence occurred within 19 patients while in 3 patients no further follow-up data was available. Median time span till tumor recurrence was 8.32 months (min: 1 month; max: 19 months). Site of tumor recurrence was local recurrence (n=3), hepatic metastasis (n=4) and disseminated metastasis (n=12). Median follow-up was 15.91 months (range: 3-45 months). All demographic characteristics of the current study cohort are summarized in (Table 1.)

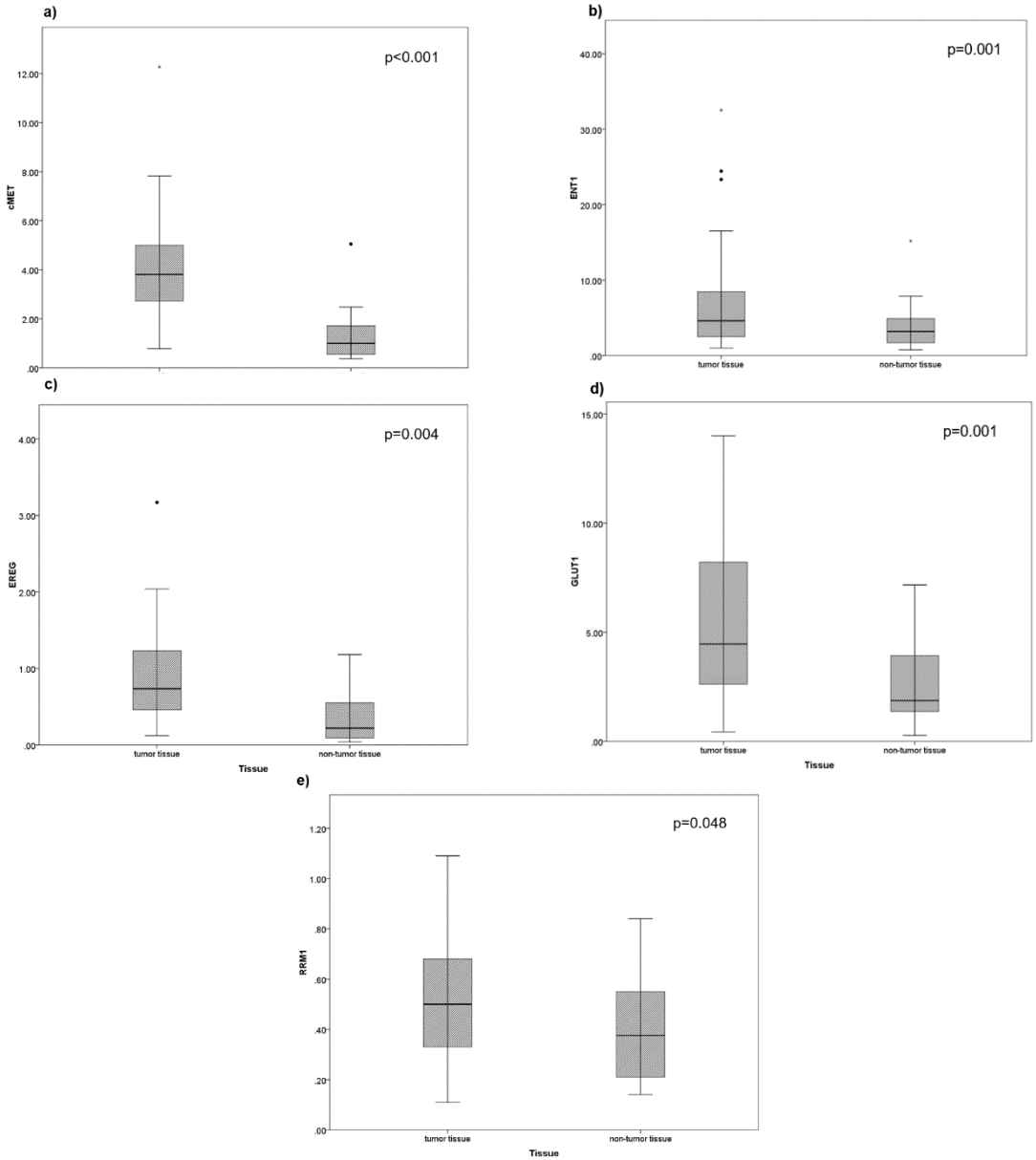

Figure 1: Tumor samples were macrodissected and mRNA analysis were performed. Afterwards, relative mRNA expression levels of both, tumor and corresponding stroma were compared. Only results from significantly different regulated genes are illustrated including a) *cMET* (p<0.001), b) *ENT1* (p=0.001), c) *EREG* (p=0.004), d) *GLUT1* (p=0.001) and e) *RPM1* (p=0.048).

II Chemotherapy and surgery

The majority of patients received chemotherapy as adjuvant therapy within a multimodal treatment concept with radical surgery. Radical surgery combined with/without adjuvant chemotherapy was performed in 26 patients. Adjuvant chemotherapeutic treatment subdivided as

follows: 15 patients underwent gemcitabine monotherapy while gemcitabine in combination with erlotinib was applicated in 3 patients. In 5 patients no chemotherapy was applied. No data was available for 3 patients.

Depending on the tumor localization different surgical procedures were performed. Pancreaticoduodenectomy (Whipple procedure) took place in 2 patients while pylorus-sparing pancreaticoduodenectomy according Traverso-Longmire was done in 11 patients. Six patients underwent distal pancreatectomy and another 6 patients received total pancreatectomy. No information considering the performed surgical procedure was given for one patient. Complete (R0) resection was archived in 18 patients. In 7 patients, complete resection was not successful resulting in R1 status (no information for one patient). Median number of resected lymph nodes was 25.73 (range: 10-66). Pathological tumor stage was pT2 in 1 and pT3 in 25 patients while the nodal status was pN0 in 7 and pN+ in 19 patients.

III mRNA Expression of target genes

Not all samples were sufficient in quality and quantity for microdissection and mRNA analysis. Therefore, selected number of usable results per target marker within tumor tissue scattered as follows: *c-MET*: n=25; *ENT1*: n=21; *EREG*: n=20; *GLUT1*: n=25 and *RRM1*: n=21. Within the stroma, the detectable contribution was *c-MET*: n= 19; *ENT1*: n=21; *EREG*: n=14; *GLUT1*: n=20 and *RRM1*: n=14.

IV mRNA expression in tumor versus stromal tissue

The quantitative mRNA expression of these genes within tumor compared to circumferential pancreatic stroma demonstrated significant higher levels of *c-MET* (p<0.001), *ENT1* (p=0.001), *EREG* (p=0.004), *GLUT1* (p=0.001) and *RRM1* (p=0.048) (see Figure 1).

V c-MET and GLUT1 are associated with poor prognosis

When statistically evaluated for the best cut-off, patients with high (>5.00) *c-MET* expression in the tumor tissue had a worse overall survival (p<0,003). Similarly, high expression of *GLUT1* (>6.57) is significantly associated with poorer survival (p=0.05) (see Figure 2). There was no prognostic impact of the other alternated mRNA expressions on the patients' survival (data not shown). The intratumoral *c-MET* mRNA-expression was not associated with locally advanced pN-category (p=0.318). Furthermore, we found no significant correlation between higher numbers of lymph node metastases and the patients' postsurgical outcome (p=0.446).

Discussion

Pancreatic cancer belongs to the tumor entities that are still associated with a poor prognosis. Only surgical resection provides a potential curative treatment option [1, 2]. However, most patients present in stages where complete surgical resection is not possible anymore [3]. Resistance to almost any systemic therapy is also a major challenge in the treatment of pancreatic carcinoma. Gemcitabine based chemotherapy, which has been the standard treatment for pancreatic cancer for many years, has only response rates between 5.6% and 13.3% [33]. Newer treatment regimens such as FOLFIRINOX achieve response rates in only around 30% and are associated with massive side effects in more than 50% of patients [4]. Therefore, novel therapeutic opportunities are urgently needed to improve the prognosis.

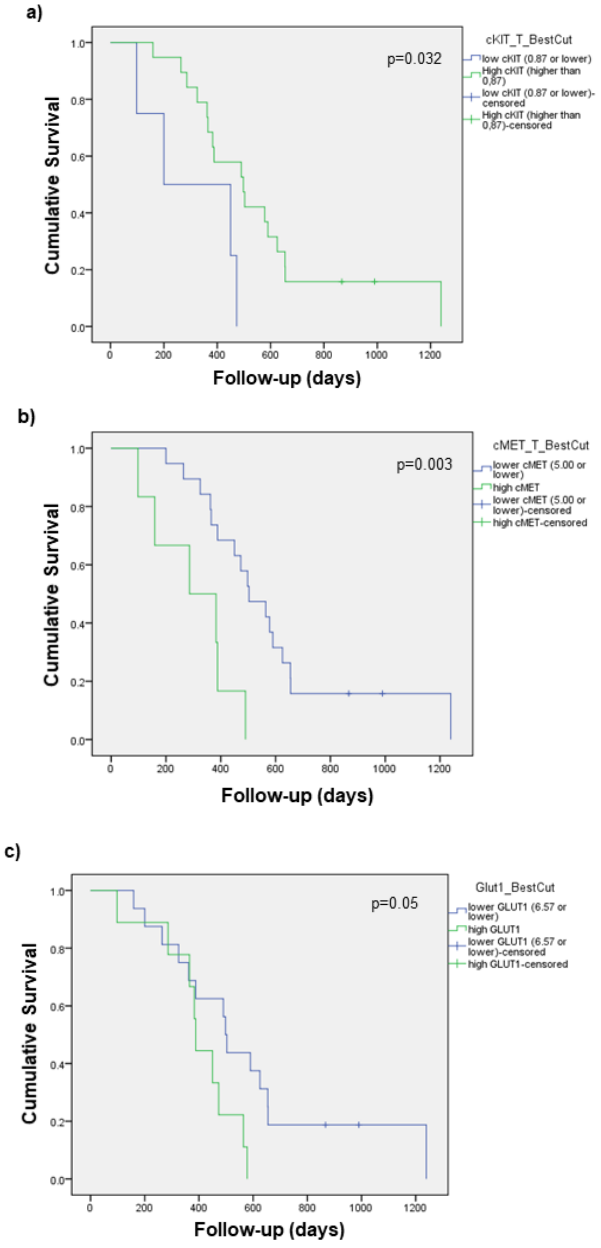

Figure 2: Correlation of the intratumoral mRNA expression and patients' survival revealed a negative correlation between high levels of a) *c-MET* (p=0.032), b) *GLUT1* (p=0.003) and c) *cKIT* (p=0.05) and poorer outcome during follow-up.

A possible influence of the genes *c-MET*, *ENT1*, *EREG*, *GLUT1* and *RRM1* in the context of pancreatic ductal adenocarcinoma and its treatment has already been described (see above) and because of the fact that pancreatic carcinoma is histologically significantly characterized by a strong stromal component , we investigated in this study *c-MET*, *ENT1*, *EREG*, *GLUT1* and *RRM1* mRNA expression patterns in pancreatic

ductal adenocarcinoma and stromal tissue in patients with clinical outcome information [34]. We could show that quantitative mRNA expression of these genes within tumor compared to circumferential pancreatic stromal tissue demonstrated significant higher levels. When statistically evaluated for the best cut-off, patients with high (>5.00) *c-MET* expression in the tumor tissue had a worse overall survival (p<0,003). Similarly, high expression of *GLUT1* (>6.57) was significantly associated with poorer survival (p=0.05). There was no prognostic impact of the other alternated mRNA expressions on the patients' survival.

Previous studies have shown an association between the resistance of pancreatic carcinoma cells and treatment with gemcitabine, which is mediated by an increased epithelial-mesenchymal transition (EMT). The genes involved in this phenotype transposition also include *c-MET* [35]. In the exocrine pancreas, there is a physiologically low expression level for *c-MET* and *HGF*. However, when proceeding to PanIN or even invasive ductal adenocarcinomas, expression of both c-MET and HGF greatly increases [11, 36, 37]. Several studies have linked activation of *c-MET* signaling pathway to phosphorylation of intracellular signaling cascades such as *PI3K/Akt*, *MAP/ERK*, or *FAK* in pancreatic cancer models, leading to tumor cell invasiveness, motility and resistance to gemcitabine therapy [12, 35, 38, 39]. Furthermore, Li et al. defined *c-MET* as a marker for pancreatic cancer stem cells with high self-renewal potential [40].

The poor response to conventional chemotherapy and the resulting low survival advantage in the treatment of pancreatic carcinoma is due, inter alia, to a high intrinsic, which means primary resistance to chemotherapy and an extrinsic, which means after repeated cycles of therapy developed secondary resistance [12]. A relationship between primary and acquired resistance to chemotherapy and activation of the *c-MET* signaling pathway has already been demonstrated for several solid tumors [41, 42]. Also, in pancreatic carcinoma, the activation of the tyrosine kinase *c-MET* is interpreted as a mechanism of this resistance development or its maintenance against chemotherapy [12]. It has been previously described that *c-MET* expression level correlates with TNM stage, lymph node status, and even after complete surgical resection with the occurrence of early distant metastasis [10, 11]. In our study the intratumoral *c-MET* mRNA-expression was not associated with locally advanced pN-category. Furthermore, we found no significant correlation between higher numbers of lymph node metastases and the patients' postsurgical outcome.

Nevertheless, inhibition of *c-MET* may increase the sensitivity to chemotherapy, particularly gemcitabine, and thus providing a promising approach for antineoplastic therapy of this devastating tumor entity. Recently, Hage and colleagues demonstrated that treatment with cabozantinib, a dual inhibitor of c-MET and VEGFR-2, increases the efficacy of gemcitabine, even when cells were resistant to this agent [12]. These results are consistent with a study by Avan and colleagues. By combining gemcitabine with the ATP-competitive c-MET inhibitor crizotinib, a significant improvement in survival was demonstrated in mice bearing primary pancreatic ductal adenocarcinoma specimen [39]. Overall, our results suggest that targeting *c-MET* could increase treatment efficacy in patients with pancreatic carcinoma. This may significantly improve current antineoplastic therapy strategies for the treatment of pancreatic cancer patients.

Conflict of Interest Statement

The authors declare no conflict of interest.

Author Contributions

Each author contributed to this paper.

REFERENCES

1. Malvezzi M, Bertuccio P, Levi F, La Vecchia C, Negri E (2013) European cancer mortality predictions for the year 2013. Ann Oncol 24: 792-800. [Crossref]
2. Siegel R, Naishadham D, Jemal A (2013) Cancer statistics, 2013. CA Cancer J Clin 63: 11-30. [Crossref]
3. Stathis A, Moore MJ (2010) Advanced pancreatic carcinoma: current treatment and future challenges. Nat Rev Clin Oncol 7: 163-172. [Crossref]
4. Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R et al. (2011) FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 364: 1817-1825. [Crossref]
5. Bladt F, Riethmacher D, Isenmann S, Aguzzi A, Birchmeier C (1995) Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. Nature 376: 768-771.
6. Jolanta Chmielowiec, Malgorzata Borowiak, Markus Morkel, Theresia Stradal, Barbara Munz et al. (2007) c-Met is essential for wound healing in the skin. J Cell Biol 177: 151-162. [Crossref]
7. Huh CG, Factor VM, Sanchez A, Uchida K, Conner EA et al. (2004) Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair. Proc Natl Acad Sci U S A 101: 4477-4482. [Crossref]
8. Blumenschein GR Jr, Mills GB, Gonzalez-Angulo AM (2012) Targeting the hepatocyte growth factor-cMET axis in cancer therapy. J Clin Oncol 30: 3287-3296. [Crossref]
9. Gherardi E, Birchmeier W, Birchmeier C, Vande Woude G (2012) Targeting MET in cancer: rationale and progress. Nat Rev Cancer 12: 89-103. [Crossref]
10. Zhu GH, Huang C, Qiu ZJ, Liu J, Zhang ZH et al. (2011) Expression and prognostic significance of CD151, c-Met, and integrin alpha3/alpha6 in pancreatic ductal adenocarcinoma. Dig Dis Sci 56: 1090-1098. [Crossref]
11. Park JK, Kim MA, Ryu JK, Yoon YB, Kim SW et al. (2012) Postoperative prognostic predictors of pancreatic ductal adenocarcinoma: clinical analysis and immunoprofile on tissue microarrays. Ann Surg Oncol 19: 2664-2672. [Crossref]
12. Hage C, Rausch V, Giese N, Giese T, Schonsiegel F et al. (2013) The novel c-Met inhibitor cabozantinib overcomes gemcitabine resistance and stem cell signaling in pancreatic cancer. Cell Death Dis. [Crossref]
13. Bauer TW, Somcio RJ, Fan F, Liu W, Johnson M et al. (2006) Regulatory role of c-Met in insulin-like growth factor-I receptor-mediated migration and invasion of human pancreatic carcinoma cells. Mol Cancer Ther 5: 1676-1682. [Crossref]

14. Hill KS, Gaziova I, Harrigal L, Guerra YA, Qiu S et al. (2012) Met receptor tyrosine kinase signaling induces secretion of the angiogenic chemokine interleukin-8/CXCL8 in pancreatic cancer. PLoS One 7: e40420. [Crossref]
15. Damaraju VL, Damaraju S, Young JD, Baldwin SA, Mackey J et al. (2003) Nucleoside anticancer drugs: the role of nucleoside transporters in resistance to cancer chemotherapy. Oncogene 22: 7524-7536. [Crossref]
16. Mackey JR, Mani RS, Selner M, Mowles D, Young JD et al. (1998) Functional nucleoside transporters are required for gemcitabine influx and manifestation of toxicity in cancer cell lines. Cancer Res 58: 4349-4357. [Crossref]
17. Spratlin J, Sangha R, Glubrecht D, Dabbagh L, Young JD et al. (2004) The absence of human equilibrative nucleoside transporter 1 is associated with reduced survival in patients with gemcitabine-treated pancreas adenocarcinoma. Clin Cancer Res 10: 6956-6961. [Crossref]
18. Zhu Z, Kleeff J, Friess H, Wang L, Zimmermann A et al. (2000) Epiregulin is up-regulated in pancreatic cancer and stimulates pancreatic cancer cell growth. Biochem Biophys Res Commun 273: 1019-1024. [Crossref]
19. WARBURG O (1956) On the origin of cancer cells. Science 123: 309-314. [Crossref]
20. Higashi K, Clavo AC, Wahl RL (1993) Does FDG uptake measure proliferative activity of human cancer cells? In vitro comparison with DNA flow cytometry and tritiated thymidine uptake. J Nucl Med 34: 414-419. [Crossref]
21. Macheda ML, Rogers S, Best JD (2005) Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. J Cell Physiol 202: 654-662. [Crossref]
22. Cornford EM, Hyman S, Schwartz BE (1994) The human brain GLUT1 glucose transporter: ultrastructural localization to the blood-brain barrier endothelia. J Cereb Blood Flow Metab 14: 106-112. [Crossref]
23. Kayano T, Burant CF, Fukumoto H, Gould GW, Fan YS et al. (1990) Human facilitative glucose transporters. Isolation, functional characterization, and gene localization of cDNAs encoding an isoform (GLUT5) expressed in small intestine, kidney, muscle, and adipose tissue and an unusual glucose transporter pseudogene-like sequence (GLUT6). J Biol Chem 265: 13276-13282. [Crossref]
24. Ito H, Duxbury M, Zinner MJ, Ashley SW, Whang EE et al. (2004) Glucose transporter-1 gene expression is associated with pancreatic cancer invasiveness and MMP-2 activity. Surgery 136: 548-556. [Crossref]
25. Olca Basturk, Rajendra Sing, Ecml Kaygusuz, Serdar Balci, Nevra Dursun et al. (2011) Glut-1 Expression in Pancreatic Neoplasia. Pancreas 40: 187-192. [Crossref]
26. Gautam A, Li ZR, Bepler G (2003) RRM1-induced metastasis suppression through PTEN-regulated pathways. Oncogene 22: 2135-2142. [Crossref]
27. Bepler G, Sharma S, Cantor A, Gautam A, Haura E et al. (2004) RRM1 and PTEN as prognostic parameters for overall and disease-free survival in patients with non-small-cell lung cancer. J Clin Oncol 22: 1878-1885. [Crossref]
28. Bepler G, Kusmartseva I, Sharma S, Gautam A, Cantor A et al. (2006) RRM1 modulated in vitro and in vivo efficacy of gemcitabine and platinum in non-small-cell lung cancer. J Clin Oncol 24: 4731-4737. [Crossref]
29. Nakahira S, Nakamori S, Tsujie M, Takahashi Y, Okami J et al. (2007) Involvement of ribonucleotide reductase M1 subunit overexpression in gemcitabine resistance of human pancreatic cancer. Int J Cancer 120: 1355-1363. [Crossref]
30. Bonner RF, Emmert-Buck M, Cole K, Pohida T, Chuaqui R et al. (1997) Laser capture microdissection: molecular analysis of tissue. Science 278: 1481-1483. [Crossref]
31. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156-159. [Crossref]
32. Gibson UE, Heid CA, Williams PM (1996) A novel method for real time quantitative RT-PCR. Genome Res 6: 995-1001. [Crossref]
33. Jie-Er Ying, Li-Ming Zhu, Bi-Xia Liu (2012) Developments in metastatic pancreatic cancer: is gemcitabine still the standard? World J Gastroenterol 18: 736-745. [Crossref]
34. Neesse A, Michl P, Frese KK, Feig C, Cook N et al. (2011) Stromal biology and therapy in pancreatic cancer. Gut 60: 861-868. [Crossref]
35. Shah AN, Summy JM, Zhang J, Park SI, Parikh NU et al. (2007) Development and characterization of gemcitabine-resistant pancreatic tumor cells. Ann Surg Oncol 14: 3629-3637. [Crossref]
36. Di Renzo MF, Poulsom R, Olivero M, Comoglio PM, Lemoine NR (1995) Expression of the Met/hepatocyte growth factor receptor in human pancreatic cancer. Cancer Res 55: 1129-1138. [Crossref]
37. Ebert M, Yokoyama M, Friess H, Büchler MW, Korc M (1994) Coexpression of the c-met proto-oncogene and hepatocyte growth factor in human pancreatic cancer. Cancer Res 54: 5575-5578. [Crossref]
38. Jin H, Yang R, Zheng Z, Romero M, Ross J et al. (2008) MetMAb, the one-armed 5D5 anti-c-met antibody, inhibits orthotopic pancreatic tumor growth and improves survival. Cancer Res 11: 4360-4368. [Crossref]
39. Avan A, Caretti V, Funel N, Galvani E, Maftouh M et al. (2013) Crizotinib inhibits metabolic inactivation of gemcitabine in c-Met-driven pancreatic carcinoma. Cancer Res 73: 6745-6756. [Crossref]
40. Li C, Wu JJ, Hynes M, Dosch J, Sarkar B et al. (2011) c-Met is a marker of pancreatic cancer stem cells and therapeutic target. Gastroenterology 141: 2218-2227. [Crossref]
41. Jung KA, Choi BH, Kwak MK (2015) The c-MET/PI3K signaling is associated with cancer resistance to doxorubicin and photodynamic therapy by elevating BCRP/ABCG2 expression. Mol Pharmacol 87: 465-476. [Crossref]
42. Ozasa H, Oguri T, Maeno K, Takakuwa O, Kunii E et al. (2014) Significance of c-MET overexpression in cytotoxic anticancer drug-resistant small-cell lung cancer cells. Cancer Sci 105: 1032-1039. [Crossref]