Expression Pattern of CDX2, Estrogen and Progesterone Receptors in Primary Gastroenteropancreatic Neuroendocrine Tumors and Metastases

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Abstract. *Background: A significant number of patients with gastroenteropancreatic neuroendocrine tumors (GEP NETs) present with metastatic disease and with unknown primary in about 15% of cases. Materials and Methods: We analyzed 163 primaries of GEP NET and 115 metastases for expression of caudal type homebox 2 (CDX2), estrogen receptor (ER), progesterone receptor (PR), somatostatin receptor 2a (SSTR2a) and Ki67. Results: PR was most often positive in pancreatic NET and only rarely in non-pancreatic NET ($p<0.001$). ER was more frequently expressed in non-pancreatic NET ($p<0.001$) and was more often positive in females than males ($p=0.019$). CDX2 was positive in all primaries of the duodenum, ileum and appendix, but was also detected in 24% of metastases with pancreatic primary. SSTR2a and Ki67 did not differ significantly between primaries and metastases. Conclusion: Our data substantiate the value of PR, ER and CDX2 in GEP NET, and steroid hormone receptors, being differentially expressed in male and female patients. Differences between primaries and metastases were small but potentially relevant.*

Neuroendocrine tumors (NETs) are rare, but an increasing incidence has been reported over time (1). A significant number of patients present with metastatic disease (2) and in about 15% of cases, the primary tumor site is not known (carcinoma of unknown primary; CUP) (3). Growth pattern, hormone production and various immunohistochemical markers are of value in identifying the primary site in such cases of neuroendocrine CUP.

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It is well-known to surgical pathologists that metastases can differ substantially from the primary tumor and it is not always possible to assign metastases to a potentially known primary by morphological comparison alone. Evidence has accumulated over the years that metastases may differ from their primaries not only in morphology but also in their immunophenotype and genotype (4-7).

The aim of the present study was to determine the expression patterns of caudal type homebox 2 (CDX2), estrogen receptor (ER), progesterone receptor (PR), somatostatin receptor 2a (SSTR2a) and Ki67 in primary tumors, and nodal and distal metastases in patients with gastroenteropancreatic (GEP) NETs.

Materials and Methods

We analyzed 163 primaries of GEP NET including 106 foregut NET (comprising 60 pancreatic NET), 48 midgut NET and nine hindgut NET. In addition, we evaluated 115 metastases (40 hematogenous metastases and 75 lymph node metastases) for expression of CDX2 (Clone EPR 2764Y; Zytomed, Berlin, Germany), ER (Clone NCL-L-ER-6F11/2; Leica, Nussloch, Germany), PR (Clone NCL-PGR-312; Leica), SSTR2a (Clone RBK046-05; Zytomed) and Ki67 (Clone MIB1 M7240; Dako, Glostrup, Denmark). All stainings were evaluated by two pathologists as a consensus score. CDX2 was scored as negative or positive; Ki67 was scored as a percentage of positively cells; SSTR2a, ER and PR were scored using the immunoreactivity score (IRS) according to Remmele and Stegner (8). An IRS of 2 or more was considered positive. The study was approved by the local Ethics Committee (no.13-093).

Results

PR was positive in 32% of all primary tumors and 18% of all metastases (Table I). A total of 28% of male and 38% of female patients had PR-positive tumors ($p=0.182$). PR expression was most often detected in pancreatic NET (77%) and it was also often positive in metastases of pancreatic NET (46%). In comparison, PR expression was infrequent in non-pancreatic NET primaries and metastases (8% and 3%,

Table I. *Expression of caudal type homebox 2 (CDX2), estrogen receptor (ER) and progesterone receptor (PR) in primary (PT) and metastatic (Met) gastroenteropancreatic neuroendocrine tumors. Metastases that could not be assigned to a specific primary site due to multiple primaries were excluded.*

	Frequency, % (n/N)					
	CDX2		PR		ER	
Primary site	PT	Met	PT	Met	PT	Met
Appendix (N=27)	100% (27/27)	100% (2/2)	11.1% (3/27)	50% (1/2)	48.1% (13/27)	0.0% (0/2)
Colon ascendens (N=3)	66.7% (2/3)	66.7% (4/6)	0.0% (0/3)	0.0% (0/6)	33.3% (1/3)	16.7% (1/6)
Colon sigmoideum	-	0.0% (0/2)	-	0.0% (0/2)	-	50% (1/2)
Ileum (N=14)	100% (14/14)	90.0% (18/20)	0.0% (0/14)	0.0% (0/22)	7.1% (1/14)	13.6% (3/22)
Duodenum (N=7)	100% (7/7)	100% (2/2)	14.3% (1/7)	0.0% (0/2)	0.0% (0/7)	0.0% (0/2)
Gallbladder (N=1)	100% (1/1)	100% (1/1)	0.0% (0/1)	0.0% (0/1)	0.0% (0/1)	0.0% (0/1)
Bile duct (N=1)	0.0% (0/1)	-	0.0% (0/1)	-	100% (1/1)	-
Stomach (N=35)	52.9% (18/34)	73.7% (14/19)	5.7% (2/35)	0.0% (0/16)	51.4% (18/35)	36.8% (7/19)
Esophagus (N=2)	50.0% (1/2)	50.0% (1/2)	0.0% (0/2)	50.0% (1/2)	50.0% (1/2)	0.0% (0/2)
Pancreas (N=57)	5.3% (3/57)	23.5% (8/34)	76.8% (43/56)	45.5% (15/33)	7.1% (4/56)	12.1% (4/33)
Rectum (N=9)	44.4% (4/9)	20.0% (1/5)	11.1% (1/9)	0.0% (0/4)	33.3% (3/9)	25.0% (1/4)
Cecum (N=4)	50.0% (2/4)	0.0% (0/2)	25.0% (1/4)	0.0% (0/2)	25.0% (1/4)	0.0% (0/2)

respectively). The difference between pancreatic and non-pancreatic NET was significant ($p<0.001$). The positive predictive value (PPV) of PR positivity and negative predictive value (NPV) of PR negativity for pancreatic origin of metastases of GEP-NET were 88% and 76%, respectively, with a sensitivity of 46% and a specificity of 97%.

ER was positive in 27% of all primary tumors and 18% of all metastases. Overall, 20% of male and 36% of female patients had ER-positive tumors ($p=0.019$). ER expression was infrequent in pancreatic NET (7%) but significantly more common in non-pancreatic NET (38%, $p<0.001$). However, in metastases, the difference was less obvious and lacked significance (12% *vs.* 21%, $p=0.287$).

CDX2 was the most frequent marker in our series and was positive in 50% of primary tumors and 54% of metastases. CDX2 was especially frequent in gastrointestinal NET primaries (75%) and their metastases (70%) in comparison to other localizations (primaries 7%, metastases 26%, $p<0.001$). The PPV of CDX2 positivity and NPV of CDX2-negativity for gastrointestinal origin of metastasis of GEP-NET were 83% and 59%, respectively, with a sensitivity of 71% and a specificity of 74%. Of note, CDX2 was positive in all primaries of the duodenum, ileum and appendix, and all metastases of duodenal and appendiceal NET. However, whereas CDX2 expression was infrequent in pancreatic primary tumors (5%), it was rather common in the metastases of pancreatic NET (24%, $p=0.012$).

The mean Ki67 index in the primary tumors was 9.8% and was not significantly higher in the metastases (12.8%, $p=0.322$). SSTR2a was positive in 74.4% of primary tumors and 68.5% of metastases ($p=0.336$).

Discussion

PR expression in NET metastases indicate a pancreatic primary. PR expression is best known in breast cancer, as well as uterine and ovarian tumors. However, it is not limited to these organs and female patients. PR expression is common in meningeoma and has been reported in gallbladder carcinomas (9), prostate cancer (10), bladder cancer (11) and many other tumor types. With regard to GEP-NET, PR was reported in pancreatic NET in 1992 by Viale *et al.* (12) and PR expression is part of an immunohistochemial algorithm for CUP-NET (13). In our study, PR expression was more frequent in pancreatic NET than reported by Viale *et al.* (12). We also found that PR expression is preserved in most cases in metastases, with high PPV, NPV, specificity and sensitivity for pancreatic origin. However, our own unpublished data indicate that PR expression can also be detected in a small number of pulmonary NETs.

ER expression is more frequent in female patients. As with PR, ER has been best studied in breast cancer and is often used in cases of CUP to potentially identify a breast, uterine or ovarian cancer as primary tumor. Data has accumulated on the expression of ER in other tumor types (14-16). The data on ER expression in GEP-NET are conflicting and differ with regard to ERα and ERβ. Whereas Viale *et al.* (12) did not detect ERα in pancreatic NET, it was reported in 40% of insulinomas (17) and 12.5% of pancreatic NET (18). Estrella *et al.* recently reported on ERβ expression in pancreatic NET, with 30%

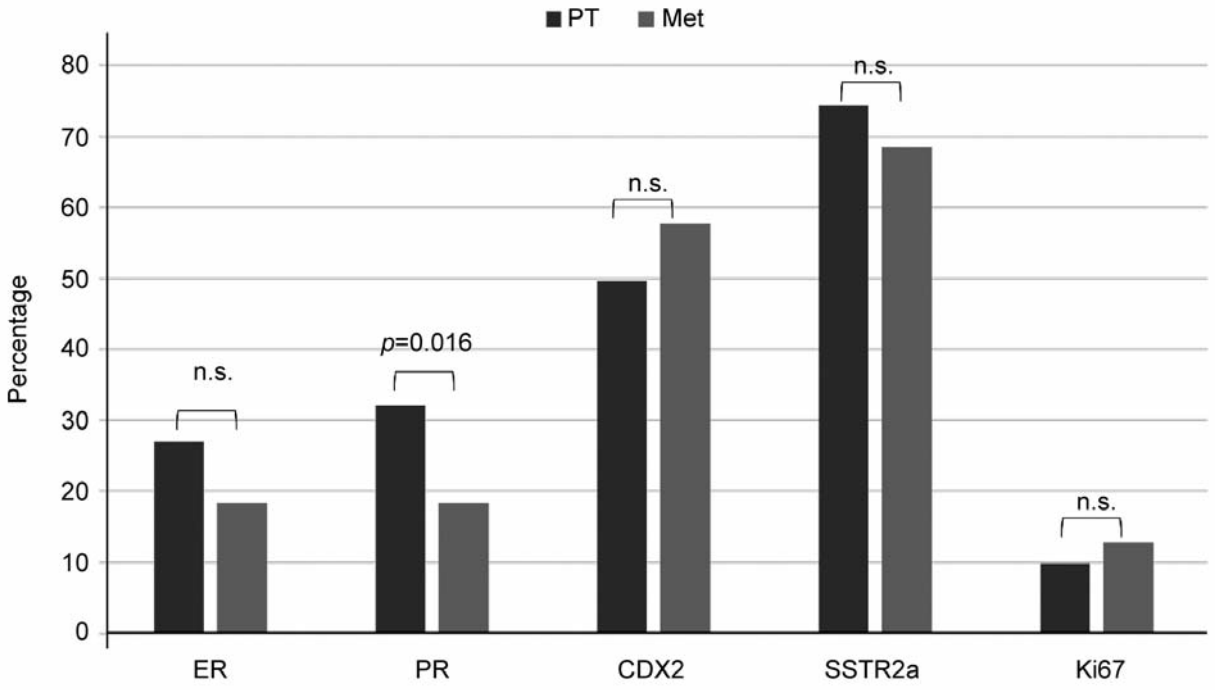

Figure 1. *Expression of estrogen receptor (ER), progesterone receptor (PR), caudal type homebox 2 (CDX2) and somatostatin receptor (SSTR2a) as a percentage of cases with positive staining in primaries (PT) and metastases (Met). Ki67 data are the mean percentage of positively stained cells. n.s. Difference not statistically significant (p>0.05).*

strong and 66% weak staining (19). In our study, we found ERα staining (IRS ≥2) in a small number of pancreatic NETs (7%) but significantly more often in non-pancreatic NETs (38%, $p<0.001$). Moreover, ERα expression was more common in female patients. It is tempting to speculate whether hormonal treatment may influence the clinical course of ER-positive NET and whether these tumors exhibit different biological behavior in male, and pre- and postmenopausal female patients. Due to the relatively small number patients in our study, further studies are needed to clarify this point.

CDX2 can be expressed in metastases of pancreatic NET. The transcription factor CDX2 is a major regulator of gut development and homeostasis (20). In our series, CDX2 was expressed in all NETs of the duodenum, ileum and appendix. These data are in line with those of Barbareshi *et al*. (21). Although CDX2 expression in NET metastases has a sensitivity of 71% and a specificity of 74% for gastrointestinal origin, it is of note that metastases of pancreatic NET were CDX2-positive in 24% of cases in our series.

Primary vs. metastases. Differences between primaries and metastases on a morphological, immunophenotypical and molecular level are well-documented for different entities (4-7). In our study, the differences between the NET primaries and the metastases were small (Figure 1). Regarding all tumors, only PR expression differed significantly between primaries and metastases. For all other markers, the differences were not significant. Of note, proliferation (Ki67) was not significantly higher in the metastases, and the expression of SSTR2a was also very similar in the primaries and the metastases (74.4% and 68.5%, respectively). Therefore, our data do not support the idea of aggressive dedifferentiation at the metastatic site (22). However, in pancreatic NET, the immunophenotype may change substantially in the metastases (*e.g.* CDX2 expression) and may hamper the identification of the primary.

In conclusion, our data substantiate the importance of PR, ER and CDX2 as additional markers in GEP-NET. Moreover, we showed that steroid hormone receptors are differentially expressed in male and female patients. The potential clinical and therapeutic relevance of this remains to be elucidated. Differences between primaries and metastases were small but potentially relevant in our series.

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