Immunohistochemical study of Aurora kinase B proves association with differentiation and expression of crucial progression markers in gastroenteropancreatic neuroendocrine neoplasms

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Objective

Gastroenteropancreatic neuroendocrine neoplasm (GEP-NENs) are rare and heterogeneous in their tumor biology. Therapeutic options to prevent growth and dissemination are still not satisfactory. As shown previously, survivin and aurora kinases (members of the mitotic chromosomal passenger complex) play a role in cell cycle progression [1]; FOXM1 is a transcription factor that regulates G2/M progression and is associated with grading and metastasis in GEP-NENs [2]. Aurora kinases, survivin and Ki-67 have been described as transcriptional targets of FOXM1. Here, we immunohistochemically analyzed this protein network as potential tumor markers.

Methods

Tumor tissues from 78 patients were studied immunohistochemically with AIM 1 (Aurora kianse B) antibody (>5%: positive) and correlated by the formerly established immunohistochemical analysis of survivin [3]. Additional 28 tissues were studied with anti-FOXM1-antibody and further 22 with anti-STAT3-antibody. The expression pattern was correlated with follow up data such as tumor progression, time of death and cause of death.

Results

The immunohistochemical analysis of Aurora kinase B revealed an association with survivin, as both nuclear scores were positively correlated (p=0.000). We further found associations with the cytosolic localization of STAT3. Aurora kinase B-expression was related to high FOXM1 expression (p=0.05). In accordance with the strong association of survivin/FOXM1 expression with grading and differentiation, we found cytosolic Aurora kinase B almost exclusively in G1/G2 tumors, nuclear Aurora kinase B expression in G3 tumors (both: p=0.000).

	Grading			
Localization	**1 Ki67 <2**	**2 ki67 3-20**	**3 Ki67 >20**	**Total**
Foregut (gastric)	2	2	1	5
Foregut (duodenal, pancreatic)	12	8	1	21
Midgut (without appendix)	21	11	1	33
Midgut (with appendix)	6	0	0	6
Hindgut	3	2	7	12
CUP	1	0	0	1
Total	45	23	10	**78**
Metastatic status				
M0	21	7	1	29
M1	14	11	8	33
Total	35	18	9	**62**

Figure 4: *Clinicopathological data of included patients*

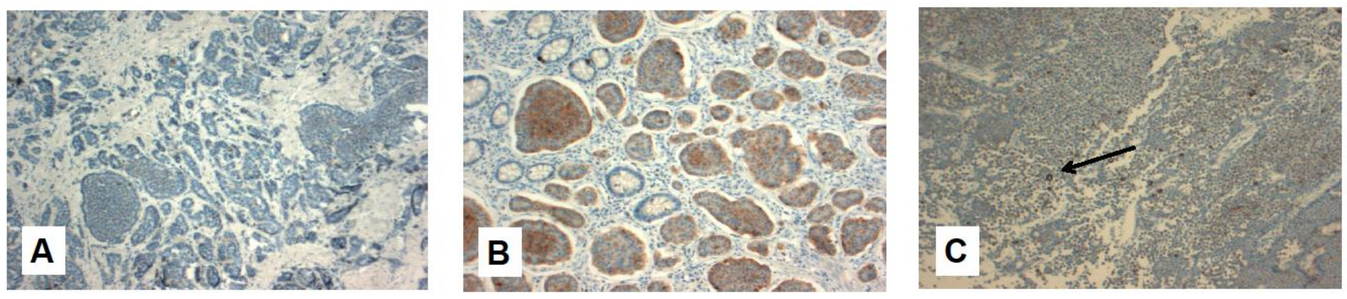

Figure 1: *Immunohistochemical staining of NENs demonstrating overexpression of Aurora kinase B: Subcellular expression revealed cytoplasmic localization in a G2 tumor of the ileum, Ki>2% (B), nuclear expression (C, arrow) in a G3 tumor of the ileum, Ki>20% and negative expression in another G2 tumor of the ileum (A) Bar = 50 µm.*

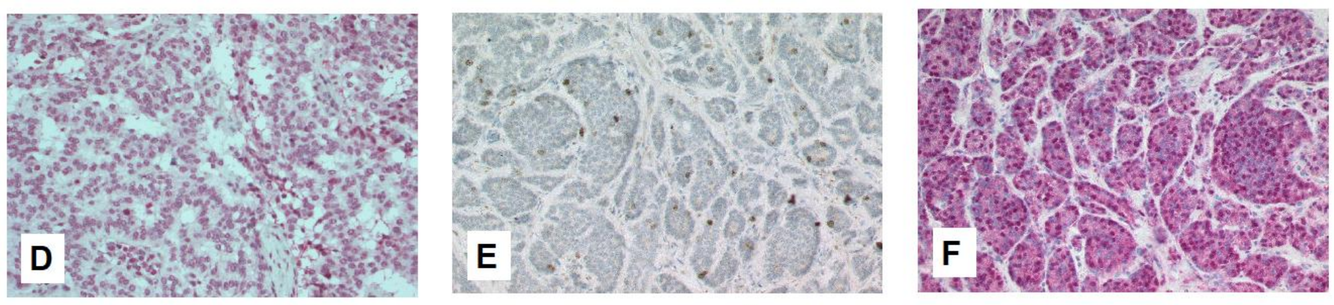

Figure 2: *Immunohistochemical staining demonstrating nuclear immunoreactivity: nuclear FOXM1 in G2 well differenciated gastrinoma, ki67 5% (D), nuclear survivin in G2 tumor of the ileum, Ki67 15-20% (E) and nuclear STAT3 in poorly differenciated gastric G3 NEC, ki67 30-40% (F) Bar = 50 µm.*

Parameter	**Survivin nuclear**	**STAT3 cytoplasmic**	**FOXM1 expression**	**Differ-entiation**	**Tumor size**	**Function-ality**
Aurora B nuclear immuno-reactivity	P=0.000 N=78	inversely P=0.023 N=22	P=0.05 N=28	P=0.000 N=78	P=0.001 N=55	inversely P=0.016 N=58
Aurora B cyto-plasmic immuno-reactivity	n.s. N=78	P=0.001 N=22	n.s. N=28	inversely P=0.000 N=78	n.s. N=55	n.s. N=58

Figure 3: *Relevant results of our immunohistochemical analyses: 78 NEN samples were stained for AIM 1 (Aurora kinase B) and survivin. Lower numbers of available tissue were additionally studied for STAT3- (n=22) and for FOXM1 expression (n=28). P-values determined by dichtiomiziation and Fisher's exact test.*

Conclusion

Our study shows that the expression of Aurora kinase B is associated with differentiation, progression and aggressiveness of GEP-NENs. We could demonstrate strong association of Aurora kinase B, FOXM1 and survivin with grading and the Ki67 proliferation status. Therefore this set of markers should be evaluated prospectively in order to better define subtypes of neuroendocrine tumors, especially in the heterogenous G3 tumor group (G3 NEN vs G3 NEC). Moreover, with this work we speculate that regulators of the G2/M cell cycle transition could be generally interesting as new targets to individualize therapeutic strategies in this tumor entity in the future.

[1] ZM447439, a novel promising aurora kinase inhibitor, provokes antiproliferative and proapoptotic effects alone and in combination with bio- and chemotherapeutic agents in gastroenteropancreatic neuroendocrine tumor cell lines. Georgieva et al. Neuroendocrinology , Vol. 91, No. 2, 2010
[2] FOXM1: A novel drug target in gastroenteropancreatic neuroendocrine tumors. Briest et al. Oncotarget, Vol. 6, No. 10, 2015
[3] Nuclear survivin is a powerful novel prognostic marker in gastroenteropancreatic neuroendocrine tumor disease. Grabowski et al. Neuroendocrinology, Vol. 81, No. 1, 2005

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