KRAS, KIT and TP53 mutations in mother's and daughter's gastric cardia adenocarcinomas

Stanislaw Gluszek[1,2], Dorota Koziel[1], Artur Kowalik[3], Sebastian Zięba[3], Slawka Urbaniak-Wasik[4], Andrzej Wincewicz[4], Stanislaw Sulkowski[5]

[1]Faculty of Medicine and Health Sciences, Jan Kochanowski University, Kielce, Poland
[2]Clinical Department of General Oncological and Endocrinological Surgery, Regional Hospital, Kielce, Poland
[3]Department of Molecular Diagnostics, Holy Cross Cancer Centre, Kielce, Poland
[4]Non Public Health Care Unit, Department of Pathology, Kielce, Poland
[5]Department of General Pathomorphology, *Collegium Pathologicum*, Medical University of Bialystok, Bialystok, Poland

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Address for correspondence: Dorota Koziel PhD, Jan Kochanowski University, 19 IX Wieków Kielc St, 25-317 Kielce, Poland, phone: +48 41 349 69 01/09, fax: +48 41 349 69 16, e-mail: dorota.koziel@wp.pl

Currently in gastric and gastroesophageal adenocarcinoma two genes are the focus of special concern in the perspective of target therapy: *ERBB2* (HER2/NEU) (17q) (human epidermal growth factor receptor 2 (HER2)) and *KRAS* (12p), whose amplifications can be detected with fluorescence in situ hybridization (FISH) both in primary tumors and metastases [1]. The high possibility of gene defects is raised whenever a cancer of the same organ is diagnosed in close relatives. Therefore, here we present the cases of two gastric cardia adenocarcinomas of a 57-year-old woman and her 39-year-old daughter with extensive gene profiling that goes beyond determination of only *KRAS* and *HER2* status.

We present a case report of two gastric adenocarcinomas of a 57-year-old woman and her 39-year-old daughter. These were mucocellular carcinoma of diffuse Lauren type (signet ring cell carcinoma) and poorly differentiated adenocarcinoma G3, respectively. Both of the tumors were located in ventricular cardia. The patients were subjected to Ivor-Lewis esophagogastrectomy. Maternal carcinoma of diffuse type was mucicarmine positive and was staged as pT3N1 and was ulcerated 5 × 3 cm in dimensions. The tumor macroscopically invaded the whole thickness of stomach wall and infiltrated adjacent adipose tissue without involvement of the radial resection margin. Lymph nodes from the vicinity of the hepatic artery and periaortic, mediastinal nodes were free of metastases. In microscopic evaluation mucocellular cancer invaded the tunica muscularis propria and tunica subserosa to be found in close proximity of the radial resection margin. Another poorly differentiated adenocarcinoma was clinically designated as a tumor of the esophagus and cardia of the daughter. It consisted partially of tube-like structures with mucin production (mucicarmine+) in luminal spaces. The tumor presented some doubtful HER-2 reaction but without apparent membranous immunolocation. Thus we performed chromogenic *in situ* hybridisation (CISH) to find the normal status of HER2 without amplification. The postoperative material included the whole stomach, which was 12 cm long along the lesser curvature and 17 cm long along the greater with an ulcerative, cauliflower-like 7 cm-in-diameter tumor that infiltrated the whole thickness of the tumor at the distance of 3 cm from the proximal resection margin. The tumor was diagnosed as poorly differentiated adenocarcinoma G3 pT4a N3b of the gastric cardia and distal part of esophagus that grew beyond the radial resection margins. There were carcinomatous emboli in small lymph vessels. There were 16 cancer nodal metastases in a total of 21 lymph nodes of the greater and lesser curvatures of the stomach. Morphology and immunoprofile of the studied neoplasm of the daughter suggest differentiation of the tumor in at least a few directions. Most of the tumor was composed of solid fields and nests of polymorphic cells with great cytologic atypia. There were also better differentiated areas of clear cells, acidophilic cells which were arranged in tube-like and cribriform structures.

There was focal positive immunoreactivity to chromogranin A in single cells and groups of cells which would highlight focal neuroendocrine differentiation. There were also AFP-positive granules in cytoplasm of some single tumor cells. Vimentin was strongly positive in a poorly differentiated part of the neoplasm. CK MNF 116 expression was decreased in the solid part of the tumor, while it was stronger in better differentiated areas. Additionally, we examined the status of following genes using NGS (IonTorrent – Ion AmpliSeq Cancer Panel v2 – Life Technologies, USA): *ABL1*, *EZH2*, *JAK3*, *PTEN*, *AKT1*, *FBXW7*, *IDH2*, *PTPN11*, *ALK*, *FGFR1*, *KDR*, *RB1*, *APC*, *FGFR2*, *KIT*, *RET*, *ATM*, *FGFR3*, *KRAS*, *SMAD4*, *BRAF*, *FLT3*, *MET*, *SMARCB1*, *CDH1*, *GNA11*, *MLH1*, *SMO*, *CDKN2A*, *GNAS*, *MPL*, *SRC*, *CSF1R*, *GNAQ*, *NOTCH1*, *STK11*, *CTNNB1*, *HNF1A*, *NPM1*, *TP53*, *EGFR*, *HRAS*, *NRAS*, *VHL*, *ERBB2*, *IDH1*, *PDGFRA*, *ERBB4*, *JAK2*, *PIK3CA* (Table I).

The mother carried mutation *TP53* (7579358 C>A p.R110L frequency 46.1%) and *KRAS* mutation of codon p.G12V (25398284 C>A, p.G12V frequency 58.7%) in tissues of the primary tumor. Similarly, *TP53* (7579358 C>A p.R110L frequency 56.2%) and *KRAS* (25398284 C>A p.G12V frequency 53.2%) mutations were detected in nodal metastasis of the primary maternal cancer. The daughter presented *TP53* (7579358 C>A p.R110L frequency 53.2%) and *KRAS* mutations (25398284 C.Ap.G12V frequency 56.2%). However, we also detected p.G565R c.1693G>A (frequency 21%), p.Q575H (c.1718G>A, frequency 20%) in the *KIT* gene and *PTEN* p.V249M (frequency 35%) in the daughter's cancer. Because of familial aggregation of the gastric cancer *CDH1* gene raw traces data were reviewed using the Integrative Genomics Viewer (IGV), but no mutation was detected (Table I).

Due to the mucinous nature of maternal mucocellular carcinoma and scantiness of malignant dispersed signet cells molecular analysis of nodal metastasis was not reliable due to low quality of the described material. We were not able to confirm the nature of the mutation, somatic or germline, because of poor quality of isolated DNA from normal tissue.

KRAS p.G12V mutation was confirmed in molecular analysis in both cases. *KRAS* mutations are not frequent in gastric cancer (5%). The presence of this mutation was correlated with mismatch repair deficiency syndrome and poor prognosis [2, 3]. However, there is still no consensus about the prognostic value of the *KRAS* mutation in the context of targeted anti-EGFR mAb [4]. A procedure of management of gastric cancer with a key role of trastuzumab and HER-2 was well-illustrated in a case report of a 49-year-old male patient by Wang *et al.* [5]. Endoscopic oligobiopsy samples served for histopathological diagnosis of gastric adenocarcinoma and for evaluation of HER2 status by immunohistochemistry and fluorescence in situ hybridization [5]. However, we did not find overexpression of HER2 in immunohistochemistry or in CISH evaluation. On the other hand, *TP53* mutation are well described and are the most frequently detected mutations in gastric carcinoma [6]. We detected *TP53* mutation p.R110L c.329G>T, which was described by Wang *et al.* [6]. This variant affects the activity of the full-length protein and causes lack of transcriptional and proapoptotic activity [7]. However, for the other *TP53* isoforms its biological consequences are unknown [7]. *TP53* mutations are found in microsatellite stable (MSS) subtype of gastric carcinoma [7]. The NGS technology also allowed us to detect p.G565R and p.Q575H mutations in the *KIT* gene. The first mutation was correlated with primary resistance to imatinib therapy in GIST [8]. However, other authors pointed out that stromal tumors of the

Table I. Mutations detected in two gastric cancers from mother and daughter by NGS

Sample	Gene ID	Position	AA Change	Frequency (%)
Mother_tumor				
	TP53	7579358 C>A	p.R110L	46.1
	KRAS	25398284 C.A	p.G12V	58.7
Mother_lymph_node				
	TP53	7579358 C>A	p.R110L	53.2
	KRAS	25398284 C.A	p.G12V	56.2
Daughter_tumor				
	TP53	7579358 C>A	p.R110L	53.2
	KRAS	25398284 C.A	p.G12V	56.2
	KIT	55593627 G>A	p.G565R	21
	KIT	55593659 A>C	p.Q575H	20
	PTEN	89717720 G>A	p.V249M	35

digestive tract had a secondary resistance mutation in *PDGFRA* exon 18 p.D842V [9]. *KIT* p.Q575H mutation has already been revealed in GIST [10], and our case of the daughter's adenocarcinoma is the second report of this mutation in the scientific literature. What is more interesting, both of the detected *KIT* mutations lie in close proximity on the same allele within the known hotspot region. More basic science is needed to resolve the oncogenic potential of these KIT mutations in both contexts as a single and as a double event. Then, imatinib could be an interesting way of new treatment for some gastric carcinoma patients.

PTEN mutations are found in EBV-positive and microsatellite instable (MSI) subtypes of gastric tumors [11]. Insertions or deletions are described as the most frequent *PTEN* mutations, which cause a frameshift mutation resulting in protein truncation [6]. Interestingly, we detected *PTEN* mutation p.V249M, and we could not find that sort of *PTEN* mutation in the published literature. Nevertheless, in the same codon Wen *et al.* described a nonsense mutation (p.V249*) in 2 patients with gastric carcinoma [12]. Overall, *PTEN* as a negative regulator of the PI3K/AKT pathway is the most frequently mutated gene (11%) after *TP53*, *CDH1* and *ARID1A* in gastric carcinoma [12].

Gastric cancer could contain small portions of chromogranin or AFP positive cells in quantities that do not allow establishment of a special histopathological type of adenocarcinoma, for example neuroendocrine and hepatoid carcinoma. In the case of the 39-year-old patient some single cells were AFP-positive. Similarly, Nishiwada *et al.* reported about advanced gastric cancer with omental multifocal involvement and extraordinary high serum AFP values and a HER-2 positive status A case of AFP-producing gastric cancer with peritoneal metastasis treated effectively with chemotherapy [13]. Interestingly, with the advent of S-1 + CDDP + trastuzumab therapy AFP level decreased rapidly to normal relatively low values and peritoneal dissemination of cancer disappeared on follow-up macroscopic inspection [13]. Thus, such a mode of therapy seems to be adequate for AFP producing HER-2 positive gastric cancer. The other therapeutic regime was proposed by Amano *et al.* [14] in a very analogous case of HER2- and AFP-expressing gastric cancer with peritoneal carcinomatosis to prove its effectiveness with a decrease of AFP level [14].

Based on our experience of 128 patients with the diagnosis of esophageal cancer or cancer of the gastroesophageal junction and poor, only 2-year long mean survival time for that group, we aimed at genetic profiling aimed at the perspective of target therapy of the mother and daughter with two presented cancers, as it is necessary to look for new methods of treatment of neoplastic conditions of similar localization besides standard chemotherapy and radiotherapy regimes [15, 16].

In conclusion, out of many screened gene structures, the mother and her daughter harbor the same *KRAS* (p.G12V) and *TP53* (p.R110L) mutations in gastric cardia adenocarcinomas, but KIT (p.G565R and p.Q575H) mutations are found exclusively in the daughter's tumor. Such findings could personalize target therapy in these tumors with the best choice of neoadjuvant treatment which is implemented in the case of *KRAS*, TP53, and KIT.

Conflict of interest

The authors declare no conflict of interest.

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