

Immunohistochemical and molecular features of primary clear cell-adenocarcinoma of the rectum, as predictive factors for individualized therapy

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Abstract

An 82-year-old male was hospitalized with rectal carcinoma that was confirmed endoscopically. Surgical resection of the rectum was performed. Intraoperative examination showed a solitary hepatic metastasis; metastasectomy was also performed. Histological examination of the surgical specimen showed mainly a trabecular arrangement of the tumor cells, alternating with tubuloglandular areas, the tumor being diagnosed in stage IV. The high-power-view examination showed that the tumor cells presented clear cytoplasm, and were diffusely marked by AE1/AE3 keratin, carcinoembryonic antigen (CEA), and CD10. A focal immunostain was also observed for keratins 7/20, vascular endothelial growth factor (VEGF), and its receptor (VEGF-R2). The tumor was proved to be microsatellite stable, presenting K-ras mutation. Based on the immunoprofile and computer scanning, metastases from clear cell renal cell carcinoma and adrenocortical carcinoma have been excluded. Based on these characteristics and the tumor stage, the final diagnosis was primary clear cell adenocarcinoma (CCA). Bevacizumab-based antiangiogenic therapy was indicated. This is the 12th primary CCA of the colorectum ever reported, and the first from Eastern Europe.

Keywords: clear cell adenocarcinoma, rectum, colorectal, therapy.

Introduction

Conventional clear cell carcinoma is known as a tumor characteristic for the kidney and the female genital organs. The presence of clear cells in carcinomas of other organs requires a very attentive differential diagnosis with a metastasis. Except for the previously nominated locations, primary clear cell carcinomas were also described in the retroperitoneum (10 cases) [1] and in other organs such as urinary bladder and urethra [2].

Primary clear cell adenocarcinoma (CCA) of the colorectal segments is an extremely rare entity, only 11

cases have been published to date, the first in 1964 and the last one in 2012; most of the cases were reported in well-developed countries, especially USA and Canada [3–12]. Our case, the 12th one, being diagnosed in Romania, is the first case from Eastern Europe (Table 1).

In this paper, we present an unusual primary CCA of the rectum. Based on its molecular and immunohistochemical features, unexplored yet in literature, a possible targeted therapy has been carried out. Differential diagnosis of this tumor was also discussed.

Table 1 – Clinicopathological features of the 12 reported primary clear cell colorectal adenocarcinomas

Authors	Clinicopathological features							
	Gender	Age [years]	Location	Relapse/survival	Tumor stage		Immunohistochemistry	
					Depth of invasion	Metastases	Positive	Negative
Hellstrom and Fisher (1964, USA) [3]	M	68	Sigmoid colon	NA	NA	NA	NA	NA
Reed <i>et al.</i> (1983, USA) [4]	NA	NA	NA	NA	NA	NA	NA	NA
Jewell <i>et al.</i> (1988, Canada) [5]	F	56	Sigmoid colon	NA	NA	NA	CEA	NA
Jewell <i>et al.</i> (1988, Canada) [5]	F	61	Sigmoid colon	NA	NA	NA	CEA	NA
Watson (1990, Canada) [6]	NA	NA	Anal canal	NA	NA	NA	NA	NA
Rubio <i>et al.</i> (1995, Sweden) [7]	M	68	Transverse colon	Survived seven months	NA	Lymph nodes, liver, lung, adrenal glands, carcinomatosis	CEA, TPA	NA
McCluggage <i>et al.</i> (2001, Ireland) [8]	M	65	Rectosigma	NA	Crossing serosa	Liver	CA125, keratin 7	Keratin 20
Braumann <i>et al.</i> (2004, Germany) [9]	M	89	Left colonic flexure	Survived two years	Muscularis	No metastases	NA	NA
Hao <i>et al.</i> (2007, China) [10]	M	37	Descending colon	Survived one year	NA	Lymph nodes	NA	NA

Authors	Clinicopathological features							
	Gender	Age [years]	Location	Relapse/survival	Tumor stage		Immunohistochemistry	
					Depth of invasion	Metastases	Positive	Negative
Soga <i>et al.</i> (2008, Japan) [11]	F	71	Sigmoid colon	No recurrences – endoscopic polypectomy	Mucosa	No metastases	Keratin 20, CD10, p53, Ki67	Keratin 7
Kanstrup Fiehn <i>et al.</i> (2012, Denmark) [12]	M	69	Sigmoid colon	No recurrences – 19 months	NA	Two lymph nodes	NA	NA
Present case (2014, Romania)	M	82	Rectum	No recurrences – five months	Crossing serosa	Four lymph nodes and liver	Keratin AE1/AE3, keratin 20, keratin 7, EMA, CEA, CD10, VEGF, VEGF-R2, MLH-1, MSH-2, Maspin	Chr, Syn, Calretinin, Inhibin, S-100, Melan-A, KMB45, c-KIT, RET, VIM, HER-2

CEA – Carcinoembryonic antigen; EMA – Epithelial membrane antigen; F – Female; M – Male; NA – Not available; NSE – Neuron-specific enolase; TPA – Tissue polypeptide antigen; VEGF – Vascular endothelial growth factor; VEGF-R – VEGF-receptor; VIM – Vimentin.

☒ Patient, Methods and Results

An 82-year-old male was admitted to hospital with symptoms suggesting rectal cancer that was confirmed at rectoscopy and on bioptic specimen. The bioptic specimen was diagnosed in another department as signet ring cell carcinoma. Due to obstructive symptoms, surgical proctectomy was performed, without preoperative chemoradiotherapy. Intraoperatively, a hepatic nodule was also identified and removed. A whole-body CT-scan was performed to exclude synchronous tumors, including renal or adrenal gland ones.

Gross examination of the surgical specimen revealed a 50×25 mm diffusely infiltrative tumor that involved the entire thickness of the rectal wall. Histopathological examination of the surgical specimen revealed a tumor that invaded the mucosa, submucosa, muscularis propria, and subserosa, breaking through the serosa and infiltrating the perirectal fatty tissue, with metastases in four of the

four regional lymph nodes (pT4N2a stage). The tumor architecture was predominantly trabecular and alveolar, with relatively abundant thin vessels, but glandular structures were also seen. The tumor cells were large, polygonal or oval-shaped, presenting well-defined cell membrane, and abundant clear cytoplasm with rare PAS-positive intracytoplasmic globules of glycogen, without Alcian Blue positivity. Pleomorphic vesicular nuclei with prominent nucleoli and minimal mitotic activity were also noted. On high power-view, it was observed that the tumor cells occurred from the dysplastic glands, the areas of transition between normal/dysplastic rectal epithelium and the tumor clear cells being well visible; this aspect excluded a metastatic tumor (Figure 1). The hepatic metastasis presented the same histological aspect as those of the primary tumor. The final tumor stage was IVa (pT4N2aM1a).

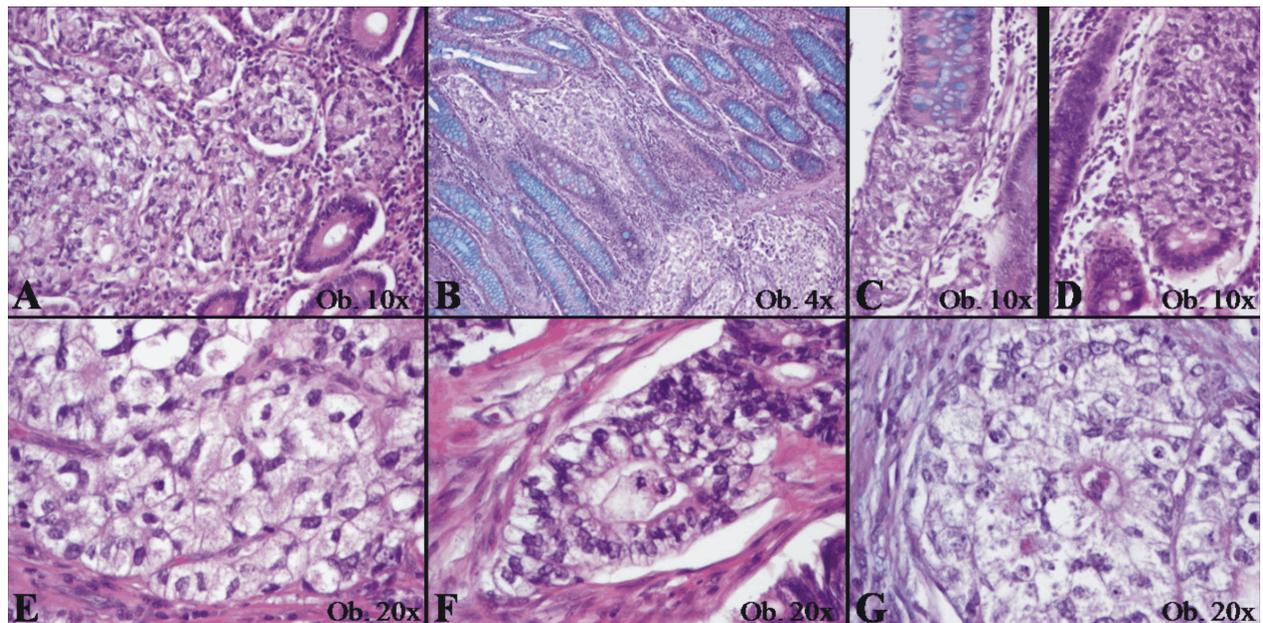


Figure 1 – Primary cell adenocarcinoma of the rectum occurs from the glandular layer (A–D) but the tumor cells do not secrete mucus (B and C); the limit between normal epithelium and clear tumor cells is well-visible in Hematoxylin Eosin (A and D) and PAS–Alcian staining (B and C). The tumor consists in proliferation of clear cells, with alveolar pattern (E) and glandular differentiation (F); few PAS-positive intracytoplasmic globules of glycogen can be seen (G).

Immunohistochemical examinations revealed that the tumor cells were diffusely marked by keratin AE1/AE3, carcinoembryonic antigen (CEA), CD10, MLH-1, and MSH-2 and presented focally positive for keratin 7/20, epithelial membrane antigen (EMA), the marker of angiogenesis vascular endothelial growth factor (VEGF), and its receptor (VEGF-R2, Flk-1). Maspin expression was revealed in the nuclei of the tumor cells, a high p53 index being also seen. No expression for chromogranin, synaptophysin, neuron specific enolase (NSE), PAX2, S-100 protein, inhibin, Melan A, HMB45, calretinin, vimentin, c-KIT, RET, or HER-2 was observed (Figure 2).

The molecular examinations, performed with Roche's

LightCycler PCR- and melting-point analysis, revealed a stable microsatellite status. K-ras gene presented mutations for both codons 12 and 13. Based on the microscopic, immunohistochemical, and molecular features, the final diagnosis was microsatellite stable/K-ras mutated primary CCA of the rectum, with nodal and liver metastases. The differential diagnosis was based on the immunoprofile presented in Table 2. Based on the liver metastases and molecular and immunohistochemical profile, a combined FOLFIRI and antiangiogenic therapy was recommended.

The postoperative course was uneventful, and to date, with neoadjuvant therapy, the patient has survived five months without any evidence of recurrence or metastases.

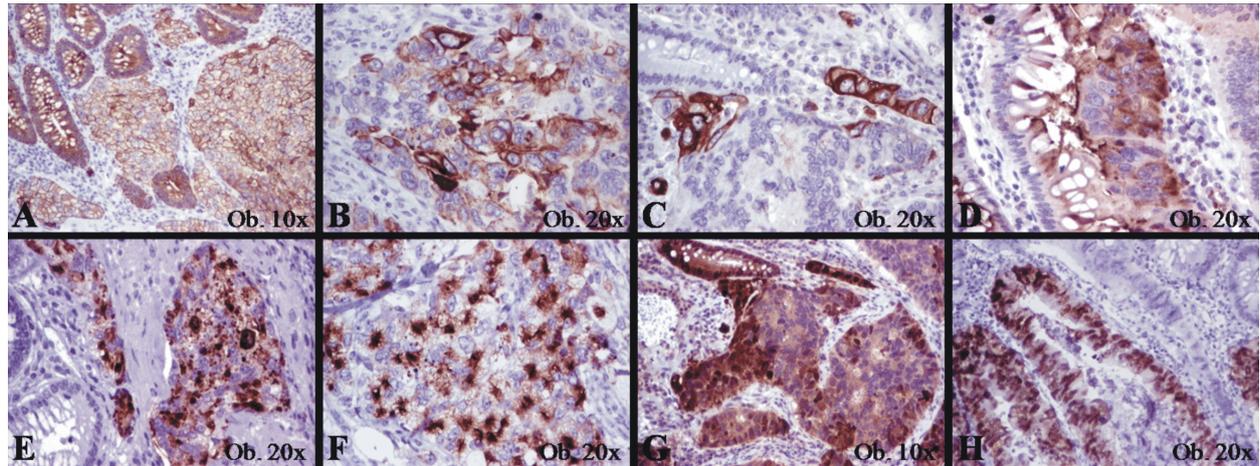


Figure 2 – Immunohistochemical findings. The tumor cells are marked by AE1/AE3 keratin (A), keratin 20 (B), keratin 7 (C), CEA (D) and CD10 (E and F). Nuclear Maspin expression (G) and a high p53 index (H) can also be observed.

Table 2 – Immunohistochemical (IHC) differential diagnosis of primary clear cell adenocarcinoma from colorectal metastatic tumors

IHC marker	Primary CCA	Primary or metastatic NET	Colorectal metastases					
			Renal cell carcinoma	HCC	Melanoma	ACC	Pheochromocytoma	Mesothelioma
AE1/AE3 keratin	+	+	±	+	-	±	±	±
Keratin 7	±	-	±	-	-	-	-	+
Keratin 20	+	±	-	-	-	-	-	-
EMA	+	+	+	+	-	±	-	+
CEA	+	±	-	±	-	-	-	-
CD10	+	-	+	+	-	-	-	-
Calretinin	-	-	-	+	±	+	-	+
Chromogranin	-	+	-	-	-	-	+	-
Synaptophysin	-	+	-	-	±	±	+	-
NSE	-	±	-	-	-	±	±	-
Vimentin	-	-	+	-	+	+	-	±
S-100	-	-	±	-	+	-	±	±
Inhibin	-	-	-	±	±	+	-	-
Melan A	-	-	-	-	+	+	-	-
HMB45	-	-	±	-	+	-	±	-
CDX2	+	±	-	±	-	-	-	-
PAX2/PAX8	-	-	+	-	-	-	-	-

ACC – Adrenocortical carcinoma; CEA – Carcinoembryonic antigen; CCA – Clear cell adenocarcinoma; EMA – Epithelial membrane antigen; HCC – Hepatocellular carcinoma; NET – Neuroendocrine tumor; NSE – Neuron specific enolase [5–13].

Discussion

Primary CCA of the colorectum was first described by Hellstrom and Fisher in 1964 as a variant of colorectal carcinoma composed by cells similar with the

physaliferous (clear) cells of chordomal tumors [3, 7, 11]. Due to its similarities with clear cell renal cell carcinoma, it was nominated 19 years later as a clear cell neoplasm or CCA [4]. Based on the literature data, synthesized in Table 1, colorectal CCA seems to arise in patients aged

around 66.6±14.1 years (ranging from 37 to 89 years). The male:female ratio is 2.3:1, all cases occurring in the distal segments, from transverse colon to anal canal [3–12]. No cases have been reported in the proximal colon segments.

Due to the rarity of this tumor, few aspects are known about its histogenesis. In kidney and female genital tract, a Müllerian origin was related with clear cell carcinoma, but it is difficult to identify its cell origin in the other organs. Some hypotheses have been stated, supposing that CCA could originate from endometriosis islands, residual embryological Müllerian nests, or Müllerian metaplasia but a genetic predisposition was also supposed [1], without strong evidences in the field. In the colon, the strong positivity for keratin 20 and CEA shows that CCA is a primary tumor of the colorectal mucosa but the presence of totipotential stem cells with further differentiation cannot be excluded. However, the well-defined limit between dysplastic rectal glands and clear cells, observed in this case, is a useful tool in differentiation between a primary and metastatic colorectal tumor. This is also in line with the authors' observation of the presence of clear cell change in some colorectal adenomas (<0.1% of cases), hypothesizing an adenoma-carcinoma sequence for CCA, similar to other histological types of colorectal tumors [5, 7, 11, 12]. Quite interesting is the immunoprofile reported for the CCA of urinary bladder and urethra, its positivity for keratin 20, CA125, and P504S (alpha-methylacyl-Coa racemase – AMACR), and p63 negativity, similar to gastrointestinal carcinomas [2], suggesting a common non-urothelial origin of CCA, independently by its location.

However, differential diagnosis of primary CCA of the colorectum is difficult to be done and is mainly based on the immunoprofile of the tumor. It should take into account the neuroendocrine tumors and also metastases from renal cell carcinoma, adrenal gland tumors, melanoma, and even mesothelioma [3–14]. The immunoprofile of these lesions was synthesized in Table 2. In females, metastases from serous ovarian carcinoma (that displays positivity for WT1 and keratin 7 and is negative for keratin 20) and cervical or endometrial carcinomas should also be taken into account [1]. Synchronous clear cell carcinoma of the kidney and colorectal adenocarcinoma was also reported, as a challenging diagnosis [15].

In the 11 cases presented in literature, the aggressive behavior of CCA was emphasized. In all of them, post-operative adjuvant chemotherapy was performed for a typical colorectal carcinoma, based on the tumor stage. In the present case, diagnosed in stage IVa, a combined FOLFIRI and Bevacizumab-based antiangiogenic therapy was indicated. Because K-ras mutation was identified, the anti-epidermal growth-factor drugs have been excluded. HER-2 negativity also excluded a HER-2 gene amplification and subsequently, a possible therapeutic response for Trastuzumab. Nuclear expression of Maspin and high p53 index confirmed that the tumor is aggressive but at the same time, was indicative of response to 5-Fluorouracil-based therapy [16]. Moreover, VEGF and VEGF-R2 positivity, correlated with c-KIT and RET negativity,

helped us to base the therapy on Bevacizumab, an anti-VEGF drug, and to exclude the antiangiogenic tyrosine kinase inhibitors, such as Pazopanib, usually used to treat metastatic renal cell carcinomas [17].

✚ Conclusions

The management of this case, based on the literature data and the molecular profile of the tumor, shows that targeted therapy can be adapted even in unusual tumors such as primary CCA of the colorectum.

Acknowledgments

This work was partially supported by the University of Medicine and Pharmacy of Țirgu Mureș, Romania, team research projects frame: POS-UMFTGM-CC-13-01-V01 No. 15/2013, and Studium Foundation. The English language manuscript was polished by SPI Global Professional Editing Service.

References

- [1] Wutankal R, Lawrence A, *Are oestrogens and genetic predisposition etiologic factors in the development of clear cell carcinoma of the retroperitoneum?* Med Hypotheses, 2013, 80(2):167–171.
- [2] Sun K, Huan Y, Unger PD, *Clear cell adenocarcinoma of urinary bladder and urethra: another urinary tract lesion immunoreactive for P504S*, Arch Pathol Lab Med, 2008, 132(9):1417–1422.
- [3] Hellstrom HR, Fisher ER, *Physaliferous variant of carcinoma of colon*, Cancer, 1964, 17:259–263.
- [4] Reed RJ, Love GL, Harkin JC, *Consultation case*, Am J Surg Pathol, 1983, 7(6):597–601.
- [5] Jewell LD, Barr JR, McCaughey WT, Nguyen GK, Owen DA, *Clear cell neoplasms of the large intestine*, Arch Pathol Lab Med, 1988, 112(2):197–199.
- [6] Watson PH, *Clear-cell carcinoma of the anal canal: a variant of transitional zone carcinoma*, Hum Pathol, 1990, 21(3):350–352.
- [7] Rubio CA, *Clear cell adenocarcinoma of the colon*, J Clin Pathol, 1995, 48(12):1142–1144.
- [8] McCluggage WG, Desai V, Toner PG, Calvert CH, *Clear cell adenocarcinoma of the colon arising in endometriosis: a rare variant of primary colonic adenocarcinoma*, J Clin Pathol, 2001, 54(1):76–77.
- [9] Braumann C, Schwabe M, Ordemann J, Jacobi CA, *The clear cell adenocarcinoma of the colon: case report and review of the literature*, Int J Colorectal Dis, 2004, 19(3): 264–267.
- [10] Hao LS, Zhou X, Zhao LH, Qian K, Zhou Y, Bu J, Wu XT, *Clear cell adenocarcinoma of colorectum: a case report and review of the literature*, Acta Gastroenterol Belg, 2007, 70(2):235–238.
- [11] Soga K, Konishi H, Tatsumi N, Konishi C, Nakano K, Wakabayashi N, Mitsufuji S, Kataoka K, Okanoue T, Mukaisho K, Hattori T, *Clear cell adenocarcinoma of the colon: a case report and review of literature*, World J Gastroenterol, 2008, 14(7):1137–1140.
- [12] Kanstrup Fiehn AM, Andersson S, Grupe Larsen L, *Primary clear cell adenocarcinoma of the colon*, Ugeskr Laeger, 2012, 174(39):2307–2308.
- [13] Gurzu S, Jung I, *Adrenocortical carcinoma: update of clinical features and diagnosis*, Global Journal of Oncologist, 2013, 1(1):42–49.
- [14] Sava I, Sava A, Șapte E, Mihailov C, Dumitrescu G, Poetă I, Sava F, Haba D, *Intraventricular metastatic clear cell renal carcinoma*, Rom J Morphol Embryol, 2013, 54(2):447–450.
- [15] Kozokic A, Surlin V, Petrovic B, Petrovic V, Prvanovic G, Beraru I, Cheregi S, *Considerations upon a case of synchronous primary malignancies: adenocarcinoma of the sigmoid and clear cell carcinoma of the right kidney*, Rom J Morphol Embryol, 2011, 52(1 Suppl):509–511.

- [16] Gurzu S, Szentimay Z, Toth E, Jung I, *Possible predictive value of maspin expression in colorectal cancer*, Recent Pat Anticancer Drug Discov, 2013, 8(2):183–190.
- [17] Maráz A, Bodrogi I, Csejtei A, Dank M, Géczi L, Kúronya Z, Mangel L, Petrányi A, Szűcs M, Bodoky G, *First Hungarian experience with pazopanib therapy for patients with metastatic renal cancer*, Magy Onkol, 2013, 57(3):173–176.

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Received: January 20, 2014

Accepted: June 18, 2014