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Histological, mucinohistochemical and immunohistochemical features of gastric signet ring cell carcinoma

Histološke, mucinohistohemijske i imunohistohemijske karakteristike *signet ring cell* karcinoma želuca

Miljan Krstić, Vuka Katić

The School of Medicine, Institute of Pathology, **Niš**

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Ključne reči:
želudac, neoplazme; mucini; histologija; imunohistohemija; fenotip.

Introduction

Gastric cancer is the fourth most common cancer and the second cause of cancer-related death worldwide [1–3]. Various types of gastric cancers differ in their epidemiology, pathogenesis, genetic profile and clinical outcome [4]. There has been the overall decline in the total incidence of gastric cancer in the last few decades worldwide, but the decline of signet ring cell (SRC) type of gastric carcinoma has been more gradual and some studies have even reported an increase of SRC type carcinoma [5]. As a result, there are different data about incidence of SRC carcinoma - it has been reported that 3.4 – 29% of patients with gastric cancer had SRC type histology and the newest data say that SRC carcinoma accounts for more than 30% of gastric carcinomas in some reported series [4–7].

Comparing with other types of gastric cancer, SRC gastric carcinoma has a tendency to involve the entire stomach and to extend directly into neighboring organs. It has the poorest prognosis, is more common in females than males and occurs at a comparatively younger age [8]. Although most researchers believe that SRC carcinoma is characterized by poor differentiation, strong invasive tendency and poor prognosis, the clinicopathologic parameters of this type of malignancy are still controversial [8–10]. Controversial reported data on gastric SRC cancer incidence, prognosis, histogenesis, metastasis, phenotipic histologic and immunohistochemical features, and confusion induced by the presence of morphologic patterns other than the conventional appearance, are the facts that require further investigations [8–15].

Classifications of gastric carcinoma

Histologic classifications

Gastric carcinomas have been classified into two main histologic subtypes by standard hematoxylin-eosin staining according to their gland-forming tendencies, intestinal and diffuse type according to Lauren [16], which essentially correspond to the differentiated and undifferentiated types, respectively, according Nakamura et al. [17].

Intestinal carcinoma was considered to be almost equal to differentiated carcinoma, and diffuse carcinoma was considered almost equal to gastric or undifferentiated carcinoma [18].

Neoplasms that contain approximately equal quantities of intestinal and diffuse components are called mixed carcinomas. Carcinomas too undifferentiated to fit neatly into either category are placed in the indeterminate category [16].

Intestinal carcinomas form recognizable glands that range from well differentiated tumors, sometimes with poorly differentiated tumor at the advancing margin. They typically arise on the background of intestinal metaplasia [19].

Diffuse carcinomas consist of poorly cohesive cells diffusely infiltrating the gastric wall with little or no gland formation. The cells usually appear round and small, either arranged as single cells or clustered in abortive, lacy gland-like or reticular formations. These tumors resemble those classified as SRC tumors in the Wored Health Organisation (WHO) classification. The mitotic rate is lower in diffuse carcinomas than in intestinal tumors. Desmoplasia is more pronounced and associated inflammation is less evident in diffuse cancers than in the intestinal carcinomas [19].

Correspondence to: Miljan Krstić, Elektronska 1/22, 18 000 Niš, Serbia. Phone: +381 18 22 65 96. E-mail: miljank@eunet.yu

With respect to histogenesis of these two types of gastric carcinoma, intestinal/differentiated type tumors have generally been considered to arise from the gastric mucosa with intestinal metaplasia and diffuse/undifferentiated type tumors from the ordinary gastric mucosa without intestinal metaplasia, and the two are considered to follow different genetic pathways during carcinogenesis [16–21].

However, recent reports have shown that gastric and intestinal phenotypic cell markers are widely expressed in gastric carcinomas, irrespective of their histological type [22–25].

World Health Organization classification

This classification is based on the predominant histological pattern. More than 50% of SRC carcinomas consist of isolated or small groups of malignant cells containing intracytoplasmic mucin. Superficially, SRC lie scattered in the lamina propria, widening the distances between the pits and glands. The tumor cells have five morphologies: nuclei push against cell membranes creating a classical signet ring cell appearance due to an expanded, globoid, optically clear cytoplasm [26]. These contain acid mucin and stain with Alcian blue at pH 2.5 (Figure 1); other diffuse carcinomas contain

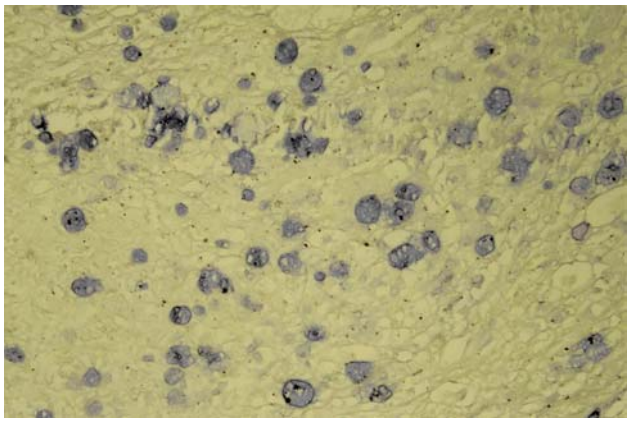

Fig. 1 – Signet ring cell gastric carcinoma – Hypersecretion of intestinal (acid) mucin (HID-AB, pH=2, 5; × 400)

cells with central nuclei resembling histiocytes, and show little or no mitotic activity; small deeply eosinophilic cells with prominent, but minute cytoplasmic granules containing neutral mucin; small cells with little or no mucin, and anaplastic cells with little or no mucins (Figure 2). These cell types intermingle with one another and constitute varying tumor proportions. SRC tumors may also form lacy or delicate trabecular glandullar patterns and they may display a zonal or solid arrangement.

Signet ring cell carcinomas are infiltrative; the number of malignant cells is comparativelly small and desmoplasia may be prominent. Histochemical stains, including mucin stains (PAS, Alcian blue, HID-AB at pH 2.5) or immunohistochemical staining with antibodies to cytokeratin, help detect sparsely dispersed tumor cells in the stroma. Cytokeratin immunostains detect a greater percentage of neoplastic cells than do mucin stains (Figure 3).

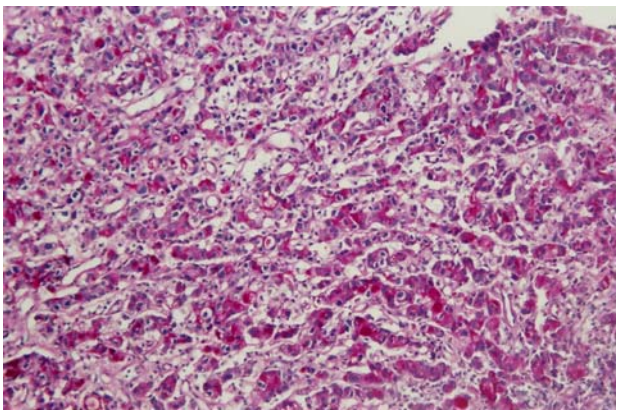

Fig. 2 – Signet ring cell gastric carcinoma – Hypersecretion of gastric neutral mucin (AB-PAS, pH=2, 5; × 200)

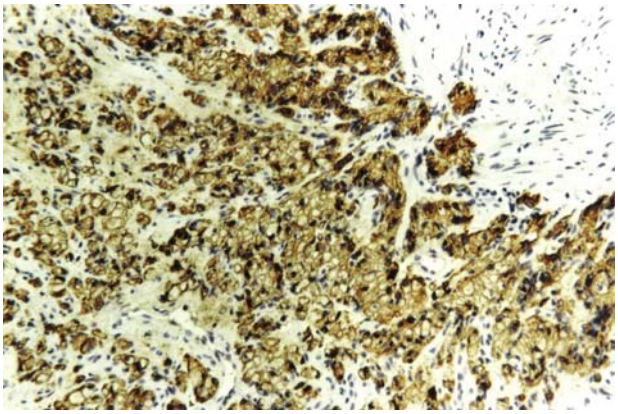

Fig. 3 – Signet ring cell gastric carcinoma – Strong diffuse expression of pancytokeratin (LSAB2; × 200)

Several conditions mimic SRC carcinoma including signet ring lymphoma, gastric mucosa associated lymphoid tissue (MALT) lymphoma, gastrointestinal stromal tumor (GIST), lamina propria muciphages, xantomas and detached or dying cells associated with gastritis [15, 26].

Phenotypic classification

Mucin histochemical and immunohistochemical methods enabled phenotypic classification of gastric cancers based on mucin expression profile.

Mucins are high molecular weight heavily O-glucosylated glycoproteins produced by secretory epithelial cells, that have many physiologic roles in normal tissues [12, 27, 28]. Specific types of mucin are individually referred to as MUC and designated with a number representing the order in which the mucin was described. Secretory mucins (MUC2, MUC5AC, MUC5B, and MUC6) act as a first-line defense as physical protective barriers for epithelial surfaces. Another subset of mucins, transmembrane type (MUC1, MUC3A, MUC3B, MUC4, MUC12, and MUC17), may serve as ligands and modulators in cell signaling [28]. Mucins have many physiologic functions as well as distinct pathologic changes in tumor and metastasis. Several protective functions as well as additional pathologic mechanisms of mucins have been proposed in cancer including protection

from host response, decreased cell adhesion, tumor invasion, and other changes in metastatic potential [28]. These two families of mucins, secretory and transmembrane, represent most of the clinically relevant mucins that have been described [27].

In gastric mucosa, two types of mucus-secreting cells exist: the surface mucous cells and gland mucous cells (which includes cardiac gland cells, mucous neck cells, and pyloric gland cells) [12].

The mucin expression pattern of gastric carcinoma is heterogeneous. It includes mucins normally expressed in gastric mucosa – gastric phenotypic markers (MUC1, MUC5AC and MUC6) and *de novo* expression of the intestinal mucin – intestinal phenotypic marker MUC2 [11, 27].

According to the expression of phenotypic markers, SRC carcinomas are classified into four differentiated phenotypes [22]. G type (tumors that are positively stained by one or more gastric phenotypic markers, but no intestinal phenotypic marker); I type (those stained by one or more intestinal phenotypic markers, but no gastric marker); GI (mixed) type (those positively stained by both gastric and intestinal phenotypic markers) and UC (unclassified type) (those stained by none of the phenotypic markers) (Figures 4–6).

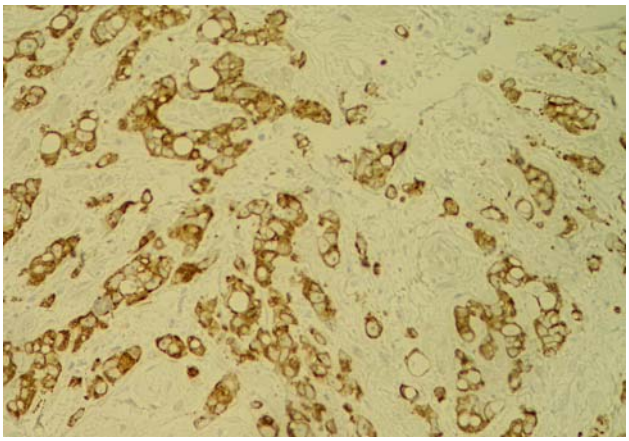

Fig. 4 – Signet ring cell gastric carcinoma – Intensive expression of MUC6 (LSAB2; × 400)

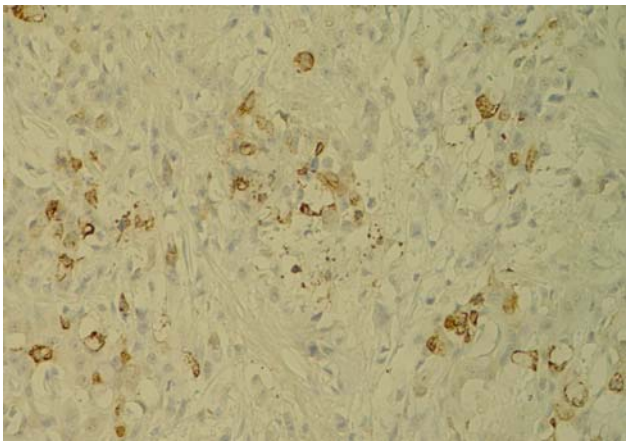

Fig. 5 – Signet ring cell gastric carcinoma – Multifocal weak expression of MUC2 (LSAB2; × 400)

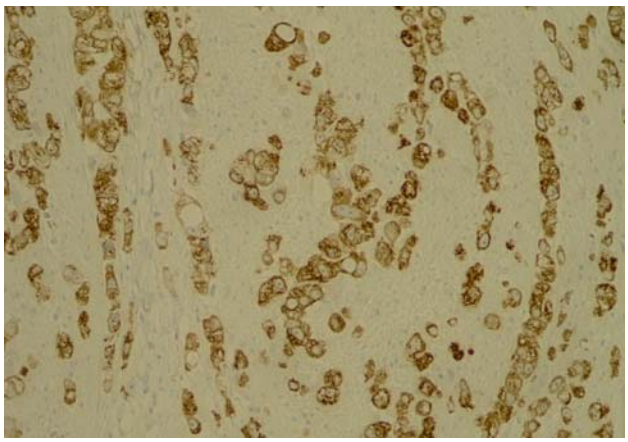

Fig. 6 – Signet ring cell gastric carcinoma – Diffuse intensive expression of MUC5AC (LSAB2; × 400)

Signet ring cell carcinoma can be easily missed on initial microscopic examination due to inconspicuousness of the tumor cells and marked desmoplasia. In addition, the WHO classification provides five morphologic patterns of tumor cells, other than the conventional appearance, inducing a great diagnostic difficulty. This different morphology, and possible similarity with epitheloid GIST, hepatoid variant of gastric carcinomas, MALT lymphomas, xantomas, and marked inflammatory and desmoplastic reaction (coupled with the inconspicuousness of the tumor cells) enter the list of diagnostic possibilities. However, the histochemistry for mucin and immunohistochemical positivity for panCytokeratin and negativity for CD117, SMA, S-100 protein, CD20 and CD45Rö, exclude this diagnostic possibility. In addition to, specific antibodies to the various kinds of mucins (MUC1, MUC2, MUC6, MUC5AC, MUC10) are also used to define gastric and intestinal phenotypes, to provide new insights in the differentiation pathways of the gastric carcinomas.

A number of clinical studies revealed the difference in biological behaviors and prognosis among patients with gastric SRC carcinoma, indicating that morphologic classification is not enough to predict the progression and outcome of this kind of gastric carcinoma, and subtype classification needs further investigations [9, 22, 25]. Our previous study showed that different phenotipic expression patterns were significantly associated with clinicopathologic parameters and prognosis of SRC carcinoma of the stomach [29–32].

Conclusion

Numerous morphologic variations of SRC carcinoma pose an important diagnostic dilemma.

Various epithelial, stromal and lymphomatous tumors, and xantomatous gastritis enter the list of diagnostic possibilities. The accurate diagnosis is essential for therapeutic and prognostic considerations.

Immunohistochemistry is “gold standard” for SRC carcinoma diagnosis.

Examination of phenotype expression may be useful evidence for further classification and prognostic prediction in gastric SRC carcinomas.

R E F E R E N C E S

1. *Stewart BW, Kleihues P,* editors. World Cancer Report. ARC World Health Organization, International Agency for Research on Cancer. Lyon: IARC Press, 2003.
2. *Kelley JR, Duggan JM.* Gastric cancer epidemiology and risk factors. J Clin Epidemiol 2003; 56(1): 1–9.
3. *Japanese Gastric Cancer Association.* Japanese Classification of Gastric Carcinoma. 2nd English Edition. Gastric Cancer 1998; 1(1): 10–24.
4. *Henson DE, Dittus C, Younes M, Nguyen H, Albores-Saavedra J.* Differential trends in the intestinal and diffuse types of gastric carcinoma in the United States, 1973–2000: increase in the signet ring cell type. Arch Pathol Lab Med 2004; 128(7): 765–70.
5. *Otsuji E, Yamaguchi T, Sawai K, Takahashi T.* Characterization of signet ring cell carcinoma of the stomach. J Surg Oncol 1998; 67(4): 216–20.
6. *Yokota T, Kunii Y, Teshima S, Yamada Y, Saito T, Kikuchi S,* et al. Signet ring cell carcinoma of the stomach: a clinicopathological comparison with the other histological types. Tohoku J Exp Med 1998; 186(2): 121–30.
7. *Kim DY, Park YK, Joo JK, Ryu SY, Kim YJ, Kim SK,* et al. Clinicopathological characteristics of signet ring cell carcinoma of the stomach. ANZ J Surg 2004; 74(12): 1060–4.
8. *Marković M, Tiodorović B, Bašić H, Vukanović A, Lazarević V, Katić V.* Clinical and histochemical characteristics of the gastric signet ring carcinoma. Monduzzi Editore Bologna 1996; 597–600.
9. *Bamba M, Sugihara H, Kushima R, Okada K, Tsukashita S, Horinouchi M,* et al. Time-dependent expression of intestinal phenotype in signet ring cell carcinomas of the human stomach. Virchows Arch 2001; 438(1): 49–56.
10. *Hyung WJ, Noh SH, Lee JH, Huh JJ, Lah KH, Choi SH,* et al. Early gastric carcinoma with signet ring cell histology. Cancer 2002; 94(1): 78–83.
11. *Reis CA, David L, Carvalho F, Mandel U, de Bolós C, Mirgorodskaya E,* et al. Immunohistochemical study of the expression of MUC6 mucin and co-expression of other secreted mucins (MUC5AC and MUC2) in human gastric carcinomas. J Histochem Cytochem 2000; 48(3): 377–88.
12. *Akamatsu T, Katsuyama T.* Histochemical demonstration of mucins in the intramucosal laminated structure of human gastric signet ring cell carcinoma and its relation to submucosal invasion. Histochem J 1990; 22(8): 416–25.
13. *Shiroshita H, Watanabe H, Ajioka Y, Watanabe G, Nishikura K, Kitano S.* Re-evaluation of mucin phenotypes of gastric minute well-differentiated-type adenocarcinomas using a series of HGM, MUC5AC, MUC6, M-GGMC, MUC2 and CD10 stains. Pathol Int 2004; 54(5): 311–21.
14. *Sugai T, Habano W, Uesugi N, Jao YF, Nakamura S, Abe K,* et al. Three independent genetic profiles based on mucin expression in early differentiated-type gastric cancers--a new concept of genetic carcinogenesis of early differentiated-type adenocarcinomas. Mod Pathol 2004; 17(10): 1223–34.
15. *Gupta R, Arora R, Das P, Singh MK.* Deeply eosinophilic cell variant of signet-ring type of gastric carcinoma: a diagnostic dilemma. Int J Clin Oncol 2008; 13(2): 181–4.
16. *Lauren P.* The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 1965; 64: 31–49.
17. *Nakamura K, Sugano H, Takagi K.* Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. Gann 1968; 59(3): 251–8.
18. *Kabashima A, Yao T, Sugimachi K, Tsuneyoshi M.* Relationship between biologic behavior and phenotypic expression in intramucosal gastric carcinomas. Hum Pathol 2002; 33(1): 80–6.
19. *Sugihara H, Hattori T, Imamura Y, Noriki S, Fukuda M, Katsura K,* et al. Morphology and modes of cell proliferation in earliest signet-ring-cell carcinomas induced in canine stomachs by N-ethyl-N'-nitro-N-nitrosoguanidine. J Cancer Res Clin Oncol 1991; 117(3): 197–204.
20. *Saito K, Shimoda T.* The histogenesis and early invasion of gastric cancer. Acta Pathol Jpn 1986; 36(9): 1307–18.
21. *Tahara E.* Genetic alterations in human gastrointestinal cancers. The application to molecular diagnosis. Cancer 1995; 75(6 Suppl): 1410–7.
22. *Tian MM, Zhao AL, Li ZW, Li JY.* Phenotypic classification of gastric signet ring cell carcinoma and its relationship with clinicopathologic parameters and prognosis. World J Gastroenterol 2007; 13(23): 3189–98.
23. *Tatematsu M, Tsukamoto T, Inada K.* Stem cells and gastric cancer: role of gastric and intestinal mixed intestinal metaplasia. Cancer Sci 2003; 94(2): 135–41.
24. *Koseki K, Takizawa T, Koike M, Ito M, Nihei Z, Sugihara K.* Distinction of differentiated type early gastric carcinoma with gastric type mucin expression. Cancer 2000; 89(4): 724–32.
25. *Morohara K, Tajima Y, Nakao K, Nishino N, Aoki S, Kato M,* et al. Gastric and intestinal phenotypic cell marker expressions in gastric differentiated-type carcinomas: association with E-cadherin expression and chromosomal changes. J Cancer Res Clin Oncol 2006; 132(6): 363–75.
26. *Fenoglio-Preiser C, Carneiro F, Correa P, Guilford P, Lambert R, Megroud F,* et al. Gastric carcinoma. In: *Stanley RH, Lauri AA* editors. Pathology and Genetics of tumours of the digestive system. WHO Classification of Tumours. Lyon: IARC Press; France, 2000. p. 39–52.
27. *Nguyen MD, Plasil B, Wen P, Frankel WL.* Mucin profiles in signet-ring cell carcinoma. Arch Pathol Lab Med 2006; 130(6): 799–804.
28. *Jass JR, Walsh MD.* Altered mucin expression in the gastrointestinal tract: a review. J Cell Mol Med 2001; 5(3): 327–51.
29. *Katić V.* Pathology of the stomach. Beograd: Medicinska knjiga; 1989. (Serbian)
30. *Jačić S, Katić V, Jančić SI, Stamenković I, Veličković Lj, Lazarević V.* Characteristics of gastric-type adenocarcinomas. Arch Oncol 2001; 9(suppl 1): 60–1.
31. *Krstić M.* Histologic and mucinohistochemical features of singet ring cell gastric carcinoma [thesis]. Niš: Medical School; 2006. (Serbian)
32. *Krstić M, Katić V.* Diagnostic dilemma of signet ring cell carcinomas of the stomach. Materia medica, 2006; 22(2): 65–6.

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