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Gastric Adenocarcinoma: Study of the Immunophenotypic Profile in View of the Latest Molecular Knowledge on Carcinogenesis

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Elena Guercio, Stefania Erra*

Surgical pathology Department Santo Spirito Hospital, Italy

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ABSTRACT

In Europe, there are about 140,000 incident cases for year of gastric adenocarcinoma, with over 100,000 deaths. Patients in an advanced stage of disease undergo pharmacological neo-adjuvant therapy. In addition to chemotherapy, it is possible to administer target therapies, with specific action for the molecular target expressed on the surface of neoplastic cells. In gastric carcinoma, the identified molecular target is the HER2 protein. In the present article, it was evaluated the molecular arrangement of the nuclear mismatch repair proteins, in light of the morphological similarities of gastric tumors with the colon ones. It can be supposed that the deficit of mismatch repair proteins could represent a determining factor for gastric carcinogenesis, as observed in some intestinal tumors.

INTRODUCTION

Gastric carcinoma accounts for 23% of neoplasms; with 192,000 new cases for year. It represents the fifth most common form of cancer in Europe. The tumor has higher incidence in older patients: about 60% of patients are over 65 years of age[1].

Most of gastric cancers are adenocarcinomas. According to Lauren's classification[2], which concerns the histological characteristics of the tumor, gastric adenocarcinoma is distinguished in "intestinal" and "diffuse". The development of the intestinal subtype includes the initial intestinal metaplasia, which can progress into dysplasia and, therefore, into carcinoma. The diffuse subtype, also known as "signet ring cells carcinoma", derives from mutations in a single cell that is part of the gastric glands, which can proliferate and begin the invasive process towards the lamina propria; characteristic of this tumor is the peculiar cellular morphology, in which the nucleus is pushed in a peripheral position by the mucin.

Neoplastic patients in advanced stages can't undergo surgery. They can be subjected to neo-adjuvant pharmacological therapy. Now-days, for many neoplasia chemotherapy treatments can be replaced or integrated with specific target therapies[3], whose action is specific only for the molecular cellular target against which they are directed. One of the first target molecule used for anti-neoplastic tailored therapy is directed against HER2 protein, an EGFR tyrosine-kinase trans-membrane protein overexpressed on the membrane of neoplastic cells in some breast tumors. The immunophenotypic determination of the expression of the HER 2 protein in breast cancer selects the patients that can undergo the treatment with Herceptin (Trastuzumab), the Her-2 molecular inhibitor.

In the present study, it is also investigated the expression of the "mismatch repair" proteins in the cases of tissue samples from gastric adenocarcinomas. In fact, complete or partial deletion of mismatch repair complex seems to be involved in carcinogenic processes in many solid tumors[4]. In the process implicating mutations in the genes of the "mismatch repair" proteins, cellular phenotypic and genotypic alterations occur, called "microsatellite instability" (MSI). Microsatellites are nucleotide sequences consisting of tandem repetitions of 1-6 base pairs; within these regions, the neoplastic process causes important genetic variations.

MATERIALS AND METHODS

The present study includes 20 selected cases of gastric adenocarcinoma from patients

between the ages of 47 and 88, whose sample tissues have been taken from surgical specimens analyzed by surgical pathology in Casale Monferrato Hospital, in Piedmont, between September 2011 and April 2017.

Formalin fixed and paraffin embedded samples have been stained by hematoxylin and eosin (H&E) for histopathological investigation. The most representative tumor sections have been selected for subsequent immunohistochemical investigations for the detection of HER2 antigen (Hercep Test) and of the "mismatch repair" proteins (MMR).

Hercep Test

Hercep Test is an immunohistochemical investigation that allows evaluating the expression of HER2 protein on the cell surface membrane[5]. HER2 is a transmembrane glycoprotein with tyrosine-kinase activity, having an extracellular receptorial domain and an intracellular kinase domain. It is involved in signal transduction and stimulation of mitogenic activity. The antibody used for the detection of HER2 antigen is the mouse monoclonal antibody VENTANA HER2/neu (4B5). The amplification of the gene and the corresponding overexpression of HER2 represents both a prognostic factor and a molecular target for the therapy with Herceptin. The interpretation of the expression of HER2[6 7] is performed thanks to specific threshold criteria that allow classification of the cases, with score values ranging from 0 to 3+, based on coloration patterns. The positivity score is based solely on the reactivity of plasma membrane staining and on the percentage of positive tumor cells. Cases with Hercep Test score 0 or 1+ are considered negative for Her2 amplification, while an Hercep-test score 3+ means Her-2 gene amplification. Hercep test score 2+ needs further investigations using Fluorescent In Situ Hybridization (FISH) method to detect real gene amplification[8].

Microsatellites

The proteins involved in the DNA repair system (MMR) and investigated in the present study are MLH1, PMS2, MSH2 and MSH6. Diagnostics of MMR-deleted tumors can be performed according to two methods. The less expensive one is represented by immunohistochemical investigation aimed at evaluating the expression of the four main MMR proteins in tumor tissue samples[9]. The other method is more expensive and it needs molecular laboratories. It allows detecting molecular analysis of microsatellite instability, which occurs thanks to the recognition of the length change of some standard microsatellite sequences. In the reported

study it was performed only immunohistochemical investigation.

RESULTS

Table 1

% cases	Hercep Test Score
65% (13)	0
20% (4)	1+
15% (3)	3+

Table 1 shows the distribution of results obtained from the Hercep Test. HER2 is overexpressed (3+ score) in 15% of cases. On this percentage of cases, it is possible to administer target therapy with Trastuzumab. About the remaining cases (85%) we can declare that carcinogenesis is not due to the involvement of the HER2 protein.

Table 2

% cases	MLH1/PMS2 complex	MSH2/MSH6 complex
60% (12)	+	+
40% (8)	-	+

Table 2 shows the distribution of results deriving from the investigation of the expression of MMR proteins. In 40% of cases the MLH1/PMS2 complex is deleted (figure 1A, 1C), whereas the MSH2/MSH6 complex is expressed (figure 1B, 1D); in the remaining 60% of cases all proteins are expressed. This should be interpreted by hypothesizing that a minority of gastric carcinomas (40%) are due to the deficiency of DNA repair mechanisms, independently from Her-2 protein status.

Table 3

n° cases	Hercep Test Score	Microsatellite status	
13	0	MSI	3
		MSS	10
4	1+	MSI	4
		MSS	0
3	3+	MSI	1
		MSS	2

Table 3 shows the overlapping of the previous results. Of the 13 cases with Hercep Test score 0, 3 have microsatellite instability, while 10 do not present deletions/mutations of the mismatch repair proteins (microsatellites stability, MSS); of the 4 cases with Hercep Test

score 1+, all cases have microsatellite instability; of the 3 cases with Hercep Test score 3+, only one case shows microsatellite instability. This relationship is also made clear by the graphic 1, which underlined the absence of correlation between expression of Her-2 protein and deletion of mismatch repair protein. The results obtained from the cases examined confirmed the data collected in the literature regarding histopathological correlations (that will be discussed in the next paragraphs): of the eight cases with MSI, six were classified as intestinal adenocarcinomas.

Graphic 1: Relationship between Hercep Test score and expression of mismatch protein in analyzed cases.

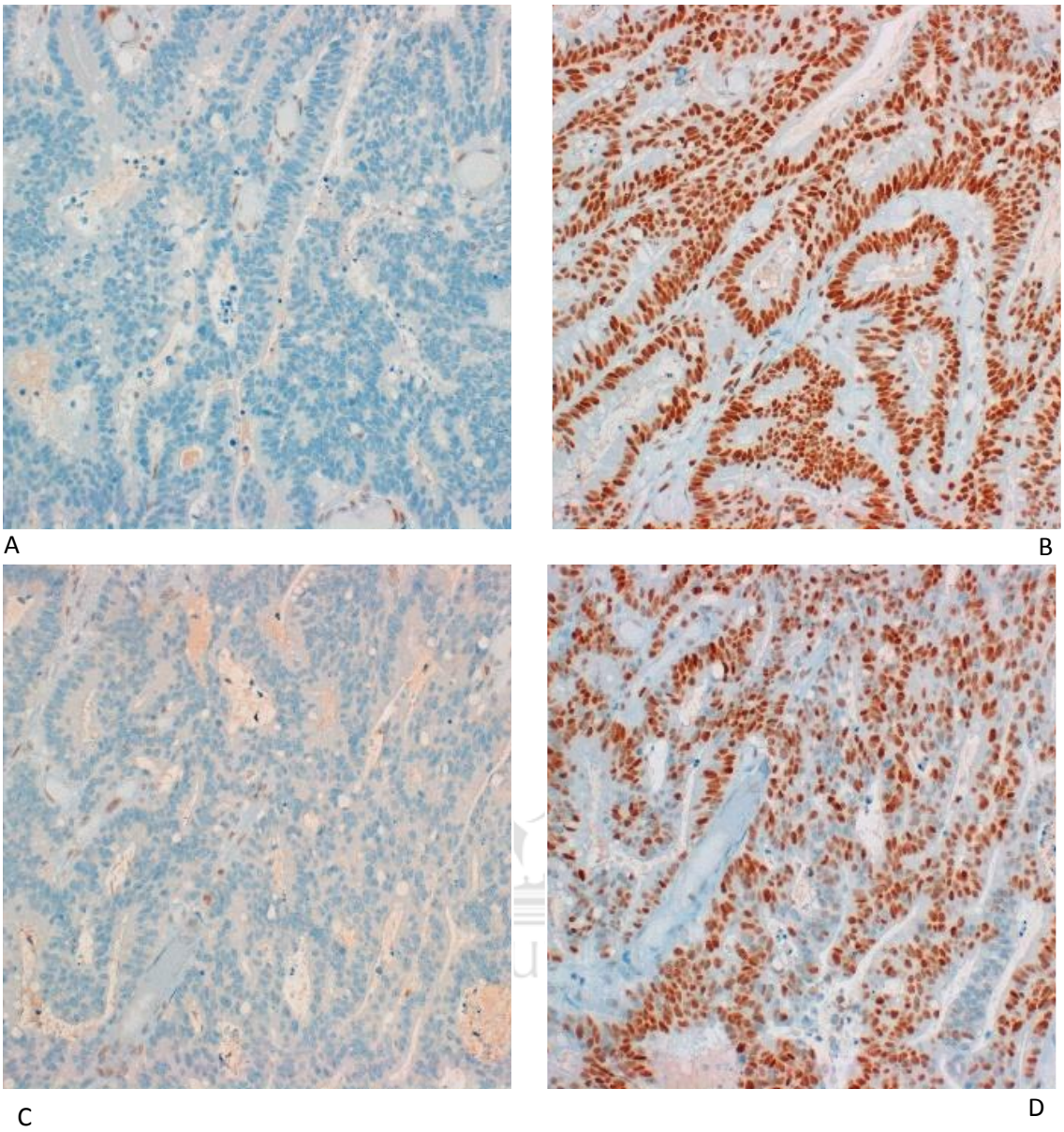

Figure 1: Immunohistochemical picture of a case of gastric cancer with deletion of mismatch repair protein (A: MLH1; B: MSH2; C: PMS2; D: MSH6)

DISCUSSION

Carcinogenesis is a multi-step and multifocal process that involves mutations of genes with an important role in cell proliferation and programmed cell death regulation. Among these genes, it seems that involvement of mismatch repair system ones could be a trigger for carcinogenesis. In 2011, the *College of American Pathologist*[10] published a paper highlighting some key features regarding carcinogenesis of many colon cancers. This document introduced the concept that microsatellites are important elements in the carcinogenesis of the tumors mentioned above. Since there are morphological similarities

between colon and gastric tumors, it has been thought that microsatellite instability and therefore a deficit in the "mismatch repair" system might have importance on the carcinogenesis of gastric tumors too. Microsatellites are nucleotide sequences consisting of tandem repetitions of 1-6 base pairs, distributed within the genome, where it is common for DNA polymerase to slip and to develop errors during DNA synthesis. The "mismatch repair" system is responsible for correcting these errors. The four main "mismatch repair" proteins that we investigated in this study are MLH1, PMS2, MSH2 and MSH6. These proteins work in tandem complexes, so MLH1 protein links PMS2 protein and MSH2 links MSH6 protein. Each complex has a specific role in the DNA repair process. When the MMR genes are deactivated by a mutation, the errors generated spontaneously during DNA replication are not corrected. Deficit of DNA repair exposes the cells to the accumulation of somatic mutations favoring carcinogenesis. As mentioned previously, in the proximity of DNA microsatellite, it is very likely that the DNA polymerase will slide, which may cause nucleotide loops, and therefore the insertion or deletion of one or more nucleotides[11]. If this event is associated with the deficiency of the mismatch repair system, there is a shortening or lengthening of the microsatellite, a condition named instability of microsatellites (MSI). Many tumor suppressors and oncogenes contain microsatellites in their coding sequence. As a consequence, the microsatellite instability condition determines the overlap of mutations in the above mentioned genes, important for the development of the tumor. To support our previous hypotheses on the similarities between gastric tumors and colon tumors, the data extracted from an article in the *World Journal of Gastroenterology*[12] shows that microsatellite instability is more common in the intestinal type cancer than the diffuse one. This observation is confirmed in the cases examined in the present study.

Further data from the literature[13] concern the prognostic impact of HER2 hyper-expression in gastric tumors. A significant prognostic datum regards survival rates: patients with HER2 gene amplification have a survival rate of around 21.4%, while patients who do not show amplification of the HER2 gene have a survival rate of 63 %, significantly higher. It follows that there is a clear association between gene amplification and the poor prognosis. HER2-neu has been recognized as co-responsible for carcinogenesis in 1/3 of gastric carcinomas. Probably, it’s a subtype of gastric malignancies that are not related to the histotype, nor to other prognostic factors such as age and anatomical-clinical staging. The overexpression/amplification of HER2-neu represents, in fact, an independent prognostic

factor of some gastric carcinomas, different for biological behavior from the HER2 not amplificated tumors and with high biological potential of greater aggression.

CONCLUSION

In conclusion, 40% of the selected cases present inactivation or deletion of the MLH1/PMS2 complex, independently from Her-2 protein status. This result seems to be related to the intestinal-type morphology of the tumors, while it is independent from clinical and pathological status. In the view to consider these tumors as linked to the lack of DNA repair system, some importance has to be given at the acquisition of this immunophenotypic profile. Identification of a tumor group with similar molecular features might help to recognize independent prognostic factors and future possibilities for effective molecular targets in personalized therapies.

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REFERENCES

1. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. European Journal of Cancer. 2013;49: 1374–1403. doi:10.1016/j.ejca.2012.12.027.
2. Lauren P. The two histological main types of gastric carcinoma: diffuse and the so-called intestinal type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 1965;64: 31-49.
3. Jomrich G, Schoppmann SF. Targeted therapy in gastric cancer. European surgery: ACA: Acta chirurgica Austriaca. 2016;48: 278–284. doi: 10.1007/s10353-016-0389-1.
4. Shokal U, Sharma P. C. Implication of microsatellite instability in human gastric cancers. Indian J Med Res. 2012; 135(5): 599–613.
5. Abrahao-Machado L. F, Scapulatempo-Neto C. HER2 testing in gastric cancer: An update. World Journal of Gastroenterology 2016;22(19): 4619–4625. doi: 10.3748/wjg.v22.i19.4619.
6. Barone C, Biffi R, De Vita F, Dei Tos A, Di Costanzo F, Doglioni C, Nicola C, Fazio N, Fiocca R, Labianca R, Maiorano E, Mottolese M, Pinto C, Rugge M, Sapino A, Scartozzi M, Sobrero A, Viale G. AIOM - SIAPEC-IAP Guidelines - Recommendations for determination of HER2 status in gastric cancer. From the AIOM - SIAPEC-IAP Working Group. Pathologica 2011; 103: 53-62.
7. Rüschoff J, Dietel M, Baretton G, et al. HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. Virchows Arch 2010;457: 299–307. doi: 10.1007/s00428-010-0952-2.
8. Anguiano A. L'ibridazione in situ in fluorescenza (FISH); principi generali ed applicazioni cliniche. Journal of Clinical Ligand Assay 2000;23: 33-42.
9. Stone J. G, Robertson D, Houlston R. S. Immunohistochemistry for MSH2 and MHL1: a method for identifying mismatch repair deficient colorectal cancer. J Clin Pathol 2001;54: 484–487.

10. Turner J. W, Baehner C. F. L, Bloom K. J, Caughron S. K, Cooper T. J, Friedberg R. C, Ho J, Monzon F. A, Wilbur D. C, Palmatier Jenkins C. Prognostic Uses of MSI Testing, College of American Pathologist 2011.
11. Leclercq S, Rivals E, Jarne P. DNA Slippage Occurs at Microsatellite Loci without Minimal Threshold Length in Humans: A Comparative Genomic Approach. Genome Biology and Evolution 2010; 2: 325–335. doi: 10.1093/gbe/evq023.
12. Velho S, Fernandes M. S, Leite M, Figueiredo C, Seruca R, Causes and consequences of microsatellite instability in gastric carcinogenesis, World Journal of Gastroenterology 2014; 20(44): 16433–16442. doi: 10.3748/wjg.v20.i44.16433.
13. Gravalos C, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. Annals of Oncology 2008; 19: 1523–1529. doi: 10.1093/annonc/mdn169.