ORIGINAL ARTICLES

Hepatocyte Antigen Expression in Subtypes of Intestinal Metaplasia of the Stomach

GA Gokhan Ocak[1], G Yildirim[2], GO Elpek[1]

ABSTRACT

Objective: *Recently, hepatocyte antigen (Hep) was introduced as a sensitive and reliable marker of intestinal metaplasia (IM). However, the distribution of Hep expression in subtypes of IM was not described.*

Methods: *We examined the expression of Hep in 58 cases of chronic gastritis associated with IM by immunohistochemical staining. Cases were classified as: 19 of IM Type I (complete) cases, 16 cases of IM Type II (incomplete) and 23 cases of IM Type III (incomplete). The distribution of Hep expression was classified into four groups according to the intensity of Hep expressing metaplastic cells: negative, low, moderate and high. We also compared expression of Hep with that of MUC-1, MUC-2 and MUC-5AC.*

Results: *Hep expression showed granular cytoplasmic staining and was specifically identified in columnar cells, but not in goblet cells. There was no significant difference between Hep expression and subtypes of IM ($p > 0.005$). However, the difference between the distribution of Hep expression among three subtypes of IM was significant ($p < 0.001$). No relationship was observed among the expression of Hep, MUC-1, MUC-2 and MUC-5AC.*

Conclusion: *Results of the present study revealed that the distribution of Hep expression is high in the majority of the complete type (Type I) IM cases, moderate in the majority of the incomplete Type II IM cases and low in all of the incomplete Type III IM cases and suggest that besides its role as a sensitive marker in IM, the evaluation of the distribution of Hep expression might be useful in the classification of IM.*

Keywords: Hepatocyte, intestinal, metaplasia, stomach

Expresión del Antígeno del Hepatocito en los Subtipos de Metaplasia Intestinal del Estómago

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RESUMEN

Objetivo: *El antígeno del hepatocito (Hep) se introdujo recientemente como un marcador sensible y confiable de la metaplasia intestinal (MI). Sin embargo, no se describe la distribución de la expresión de Hep en los subtipos de MI.*

Métodos: *Se examinó la expresión de Hep en 58 casos de gastritis crónica asociados con MI mediante tinción inmunohistoquímica. Los casos fueron clasificados como: 19 casos de tipo MI (completo), 16 casos de tipo MI II (incompleto), y 23 casos de tipo MI III (incompleto). La distribución de la expresión del Hep se clasificó en cuatro grupos según la intensidad de Hep, que expresa las células metaplásticas:*

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negativa, baja, moderada y alta. También se comparó la expresión de Hep con la de MUC-1, MUC-2 y MUC-5AC.

Resultados: *La expresión de Hep mostró tinción citoplasmática granular, específicamente identificada en las células columnares, pero no en las células caliciformes. No hubo ninguna diferencia significativa entre la expresión de Hep y los subtipos de MI ($p > 0.005$). Sin embargo, la diferencia entre la distribución de la expresión del Hep entre tres subtipos de MI fue significativa (*$p < 0.001$*). No se observó relación alguna entre la expresión de Hep, MUC-1, MUC-2 y MUC-5AC.*

Conclusión: *Los resultados del presente estudio revelaron que la distribución de la expresión de Hep es alta en la mayoría de los casos MI de tipo completo (tipo I), moderada en la mayoría de los casos MI de tipo II, y baja en todos los casos MI de tipo III incompleto. Los resultados sugieren que además de su papel como marcador sensible en MI, la evaluación de la distribución de expresión del Hep podría ser útil en la clasificación de MI.*

Palabras claves: Hepatocito, metaplasia intestinal, estómago

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INTRODUCTION

Gastric carcinoma is the second most common cancer worldwide with high mortality rates. Development of gastric carcinoma is a multistep process. It involves chronic gastritis, mucosal atrophy, intestinal metaplasia and gastric carcinoma, respectively (1). In this pathway, intestinal metaplasia (IM) is a premalignant lesion with 10-fold increased risk of gastric cancer and categorized as complete (Type I) or incomplete [Type II or III] (2).

Recent studies have shown the predictive value of identifying subtypes of IM in the risk assesment for gastric carcinoma development (2−4). Intestinal metaplasia is traditionally classified by mucin modifications confirmed by histochemical and immunohistochemical methods (5, 6).

Hepatocyte (Hep) is a cytoplasmic antigen expressed in normal hepatic parenchyma, the majority of hepatocellular carcinomas and rare carcinomas of various other tissues (7−10).

Recently, it was also introduced as a sensitive and reliable marker of IM (11−13). However, to date, the distribution of Hep expression in subtypes of IM is not described. The objective of this study was to investigate the distrubution of Hep expression in subtypes of IM.

SUBJECTS AND METHODS

Fifty-eight cases of chronic gastritis associated with IM were selected from the surgical pathology files at the Akdeniz University medical school from 2007 to 2009. Of the 58 cases, 27 were females and 31 were males. Ages of patients were from 32 to 65 years with an average of 49.8 years. The tissues had been routinely fixed in 10% formaldehyde and embedded in paraffin; 5 µm thick sections were prepared for further histochemical and immunohistochemical staining procedure.

Histochemistry: High iron diamine and alcian blue (ph 2.5) [HID-AB 2.5] stains were used to stain sulphated (brown) and acidic non-sulphated (blue) mucosubstances simultaneously, in all cases. Intestinal metaplasia was classified as follows: Type I, mature absorptive cells and goblet cells, the latter secreting sialomucins; Type II, few or absent absorptive cells, presence of columnar "intermediate" cells in various stages of differentiation secreting neutral and acid sialomucins or, occasionally, sulfomucins and/or both; and Type III, columnar "intermediate" cells secreting predominantly sulfomucins and goblet cells secreting sialomucins or sulfomucins, or both.

Immunohistochemistry: Immunohistochemical stainings against MUC-1 (1:100, neomarkers, CA, USA), MUC-2 (1:100, neomarkers, CA, USA), MUC-5AC (1:100, neomarkers, CA, USA) were performed using a streptavidin peroxidase method after an antigen retrieval using microwaves. According to mucin expression patterns, cases were classified as 19 cases of IM Type I, 16 cases of Type II and 23 cases of Type III. In addition, immunohistochemical staining against Hep (1:100, DAKO, Glostrup, Denmark) was also performed. The degree of Hep expression for each case was scored as: negative, low (between 1% and < 30%), moderate (between 30% and < 60%) and high (≥ 60%).

Statistically, the Chi-square test was performed to determine the correlation between the subtypes of IM and Hep expression status. Spearman's rank correlation coefficient was used to establish the relationship between quantitative parameters. Data are expressed as mean ± standard deviation and a p-value < 0.05 is considered significant. All statistical analyses were conducted using the SPSS 10.0 (SPSS, Chicago, IL).

RESULTS

Hep was specifically expressed in IM but not in gastric mucosa (Fig. 1). In areas of IM, only columnar cells showed cytoplasmic granular Hep positivity. Goblet cells were negative. Hep expression and its degree among three subtypes of IM are summarized in the Table. All cases of Type I IM, 14 cases (87.5%) of Type II IM and 19 cases (82.6%) of Type III

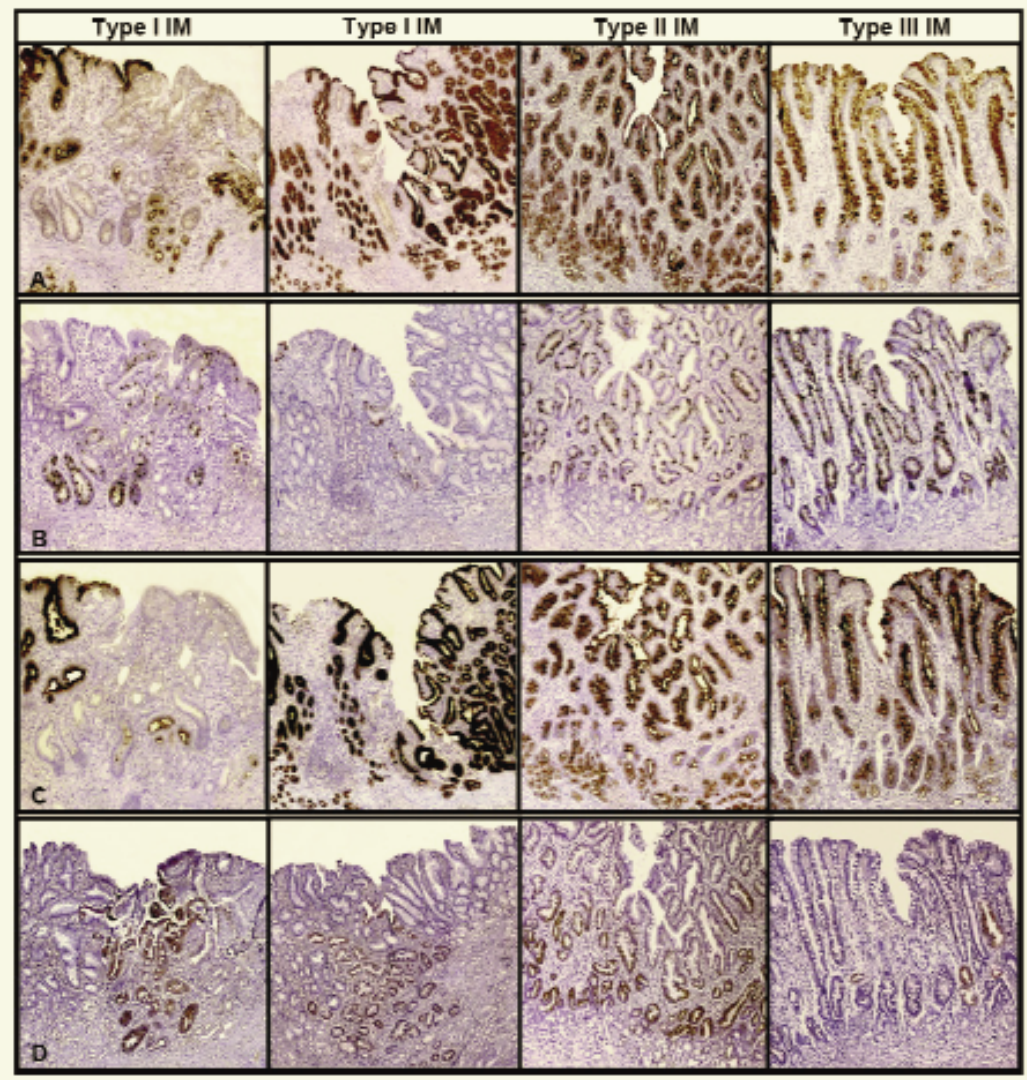

Fig. 1: MUC-1 (A), MUC-2 (B), MUC-5AC (C) and hepatocyte antigen [Hep] (D) immunohistochemical staining of serial sections from three subtypes of intestinal metaplasia (IM). Type I IM, does not express MUC-1, MUC-5AC and shows expression of MUC-2 in goblet cells. The degree of Hep expression is high and observed in all metaplastic glands. In Type II and Type III IM, MUC-1, MUC-2 and MUC-5AC are coexpressed. The degree of Hep expression is lower in Type II IM when compared to Type I. In Type III IM, the degree of Hep expression is low and limited in a few metaplastic glands.

IM showed Hep expression and subtypes of IM (> 0.05). Regarding the degree of Hep expression, in Type I IM, it was high in 16 cases and moderate in three cases. In Type II IM, the expression was moderate in a great majority of cases (10) with only four cases with high expression. In both groups low expression was not observed. Conversely, in Type III IM, low expression was observed in many cases. In the same group, moderate expression was noted in two cases and none displayed a high Hep expression. The difference between the degree of Hep expression among three subtypes of IM was significant ($p < 0.001$, r = -0.844). No relationship was observed among the expression of Hep, MUC-1, MUC-2, and MUC-5AC (Table and Fig. 2).

DISCUSSION

In this study, we observed Hep expression in 52 of 58 cases (89.65%) of IM. Normal gastric mucosa was consistently negative. Hep expression was detected in all cases of Type I (complete form of IM). Although in the incomplete IM group, two cases of Type II and four cases of Type III did not show any positivity with Hep, there was no significant difference between the presence of Hep expression and subtypes of IM ($p > 0.05$). Recently, the expression of Hep in non-neoplastic small intestinal mucosa has been demonstrated (10, 14). Based on the similarity of IM to that of intestinal mucosa, some researchers evaluated the role of Hep expression in IM. Chu *et al* (11) studied Hep expression in

Table: Hepatocyte antigen (Hep), MUC-1, MUC-2 and MUC-5AC expression in three subtypes of intestinal metaplasia (IM)

	Type I IM		Type II IM		Type III IM	
	Positive (%)	**Negative (%)**	**Positive (%)**	**Negative (%)**	**Positive (%)**	**Negative (%)**
MUC-1						
Goblet cells	5 (26.3)	14 (73.3)	14 (87.5)	2 (12.5)	14 (60.9)	9 (39.1)
Columnar cells	10 (52.6)	9 (47.4)	13 (81.3)	3 (18.8)	20 (87)	3 (13)
MUC-2						
Goblet cells	19 (100)	0	16 (100)	0	23 (100)	0
Columnar cells	0	19	9 (56.3)	7 (43.8)	2 (8.7)	21 (91.3)
MUC-5AC						
Goblet cells	4 (21.1)	15 (78.9)	7 (43.8)	9 (56.3)	11 (47.8)	12 (52.2)
Columnar cells	0	19 (100)	16 (100)	0	13 (65.5)	10 (43.5)
Hep expression						
Goblet cells	0	19	0	16	0	0
Columnar cells	19 (100)	0	14 (87.5)	2 (12.5)	19 (82.6)	4 (17.4)
Hep distribution*						
0	0	0	0	2 (12.5)	0	4 (17.4)
1	0	0	0	0	17 (73.9)	0
2	3 (15.8)	0	10 (62.5)	0	2 (8.7)	0
3	16 (84.2)	0	4 (25)	0	0	0

* $p < 0.001$

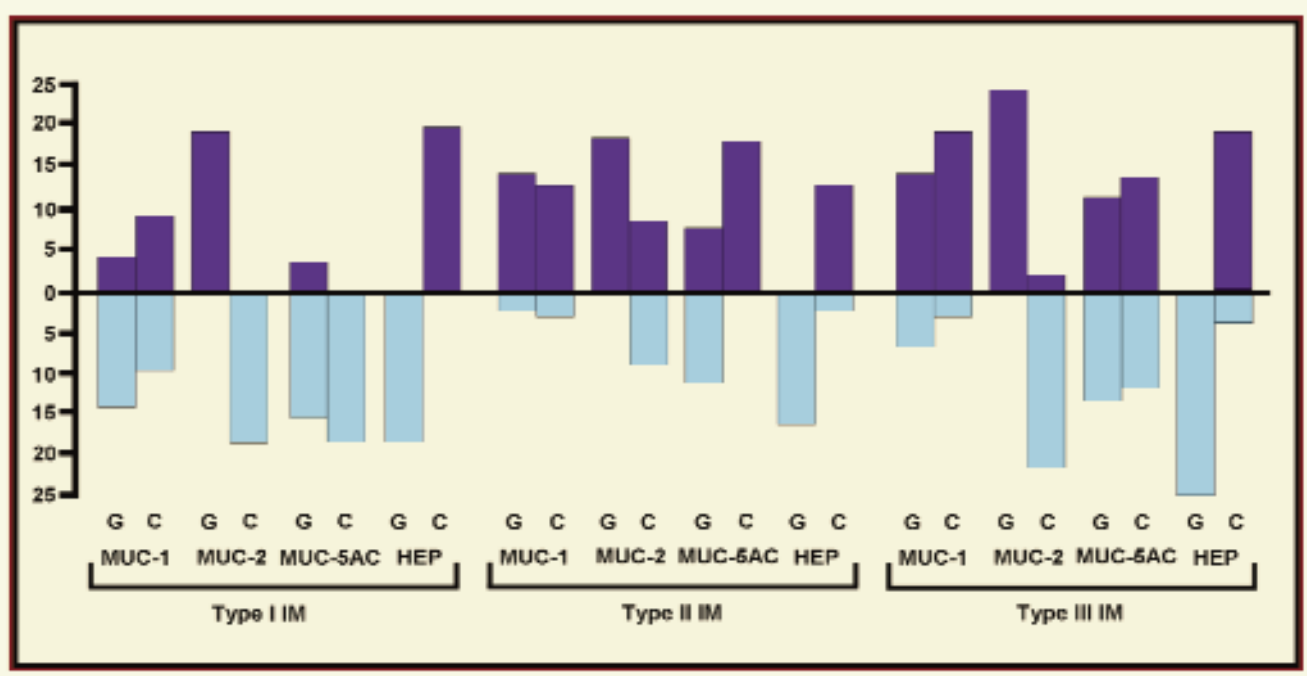

Fig. 2: Diagram representing MUC-1, MUC-2, MUC-5AC and hepatocyte antigen (Hep) expression pattern in intestinal metaplasia (IM). For each type of IM, the number of positive and negative cases for G and C cells was plotted according to immunohistochemistry. G: goblet cells, C: columnar cells.

the metaplastic mucosa of 31 cases with Barrett's oesophagus and 13 cases with gastric carcinoma and compared its expression with that of cytokeratin 7, cytokeratin 20 or MUC-2. They observed Hep expression in IM mucosa of all cases with higher specificity and sensitivity than the other markers. It was concluded that Hep immunostain may be helpful as a single diagnostic marker for IM. On the other hand, Lee *et al* (12) assessed Hep expression in 15 cases with IM of the stomach. All cases of Type I and Type III IM, but only two cases out of five Type II IM showed Hep expression. Be-

sides, Hep expresssion in incomplete forms of IM (Types II and III) was restricted to focal areas. In both studies and in our study, a common finding was the expression of Hep in the complete Type of IM, supporting the similarity of this type of metaplasia to small bowel epithelium. Regarding incomplete forms of IM, Hep expression is different among studies, warranting further investigations with larger series. However, our data support the reliability of Hep expression in the detection of IM of the stomach.

Previous studies have shown the predictive value of identifying the incomplete Type of IM in the risk assessment of gastric carcinoma (3, 4). Therefore, a subclassification of IM is valuable in order to manage these patients. Although in many cases, IM can be identified by the presence of goblet cells, it can be notoriously difficult to discern this lesion when goblet cells are inconspicuous, IM glands are hard to find and tissue samples are fragmented. Moreover, in these situations, a subclassification of IM is not possible by haematoxylin and eosin stained sections and necessitates further immunohistochemical evaluation. Recently, many studies have been addressed to discriminate the subtypes of IM by many immunohistochemical markers including Hep antigen (11−13). As Hep expression is observed in non-goblet columnar cells of IM, our data and the results of the previous studies lead us to emphasize that Hep expression might be useful in the detection of incomplete types of IM in which goblet cells are not numerous when compared to the complete type. In the present study, another worthwhile finding is the difference in the distribution of Hep expression among three subtypes of IM. While the degree of Hep expression is higher in the complete type (Type I) of IM, Hep expression is lower in incomplete IM, with only two cases with moderate Hep expression ($p < 0.05$). Although, at present, it is not possible to explain the loss of Hep expression in the incomplete forms of IM, this does not exclude the significance of our finding that showed a close association between the degree of Hep expression and the subtypes of IM.

Since its first discovery in 1993 (15), Butler *et al* (16) demonstrated that the peptid sequences of the protein immunoprecipitated by Hep is a rate-limiting enzyme of the urea cycle, carbamoyl phosphate synthetase 1(CPS1). This is not surprising for hepatocytes because in the liver, CPS1 has an integral role in urea synthesis. In contrast, the presence of CPS1 that has been established in the small bowel is confusing and at present its metabolic importance is unknown. It has been theorized that the main function of this enzyme in the small bowel may be amino acid metabolism and, possibly, the formation of nitric oxide [NO] (17, 18). Recent studies on small bowel adenomas and carcinomas revealed that in all cases of small intestinal adenomas with low grade dysplasia, Hep expression was strong and diffuse (14). The areas containing high grade dysplasia displayed a more variable pattern of immunoreactivity, whereas in adenocarcinomas Hep expression was completely lost in a great majority of cases. Although the mechanism for this alteration is not clearly known, these data demonstrate that dramatic loss of CPS1 causes Hep expression to decrease during small bowel carcinogenesis (10, 14). Therefore immunohistochemical assessment of Hep antibody may be useful in differentiating small-intestinal adenomas from adenocarcinomas. When the similarity of small bowel mucosa to IM is taken into consideration, the decrease in the degree of Hep expression in IM might be beneficial in the discrimination of incomplete forms of IM in gastric biopsies. Moreover, the loss of Hep expression during small bowel carcinogenesis leads to speculation that alteration of CPS1 might have a role in gastric carcinogenesis arising from incomplete forms of IM.

In conclusion, our data support previous data about Hep expression as a sensitive marker of IM in gastric biopsies. The frequent and high expression of Hep in Type I IM may reflect the remarkable similarity of this form of metaplasia to that of small intestinal mucosa. Moreover, our results revealed that the degree of Hep expression gradually decreases in the incomplete types (Type II and III) of IM and suggest that the evaluation of the distribution of Hep expression might be used as a diagnostic aid in the classification of IM.

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