THE IMMUNE MICROENVIRONMENT OF VARIOUS HISTOLOGICAL TYPES OF EBV-ASSOCIATED GASTRIC CANCER

Ilya Mikhailov, Natalya Danilova, Pavel Malkov

Department of Pathology, Lomonosov Moscow State University

Introduction

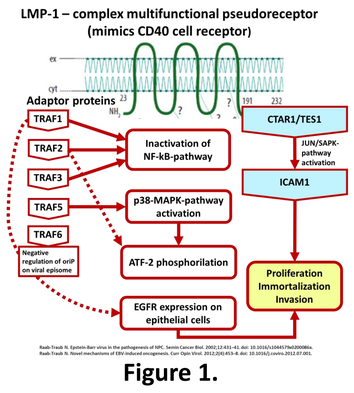


Figure 1.

EBV-associated cancer is one of the molecular subtypes of gastric adenocarcinomas classified by mutation profile analysis[1]. There is very few data on its immunohistochemical characteristics and its immune microenvironment. The distribution of immune cells might appear to be an important diagnostic criteria of poor prognosis.

LMP-1 (latent memrain protein type 1) is one of the main viral proteins; pathogenesis both lymphomas and epithelial neoplasms is associated with this protein (Fig.1).

Therefore, LMP-1 can be considered as a reliable marker for Epstein-Barr virus infection of epithelial cells including for cells of epithelial origin in the stomach.

[1]Comprehensive molecular characterization of gastric adenocarcinoma. // Nature. England, 2014. Vol. 513, № 7517. P. 202–209.

Methods

Samples of 67 gastric adenocarcinomas (surgical material) were included in this study.

Age group	Age range	Males	Females	TOTAL
Adult	From 18 to 44 years	**1**	**5**	**6**
Middle aged	From 45 to 64 years	**14**	**21**	**35**
Aged	From 65 to 80 years	**10**	**12**	**22**
80 and over	80 years over	**0**	**4**	**4**

The EBV-identification

IHC-staining for LMP-1 protein were used for EBV identification (primary mouse monoclonal antibodies Dako, clone CS.1-4). LMP-1 expression was considered as positive if out of the selected 10 fields of view, x40 increase, ratio of the cells number with LMP-1 expression to the total cells number exceeded 10% at least in one field of view. Only cytoplasmic expression of LMP-1 was considered as positive.

The Assessment of Immune Microenvironment

The study of the cellular composition of the tumor tissue infiltrate was performed by immunohistochemical staining on the markers CD4, CD8, CD68 and CD1a. For the calculation, the following quantitative method was used:

- throughout the tumor fragment **three sites** with the highest infiltration density were selected (**excluding sites of chronic inflammation, erosion and secondary necrosis**);
- in each of the three fields of view, **x20 magnification** was used to calculate the number of stained cells by two independent investigators;
- if the results of the counts differed by less than 20%, then the average number of cells in three fields of view was considered, and then this quantitative parameter was used as a characteristic of the immune microenvironment.

Importantly, the tumor tissue infiltration and **the infiltration of the gastric mucosa lamina propria** (if its were present in the investigating material) were evaluated in parallel.

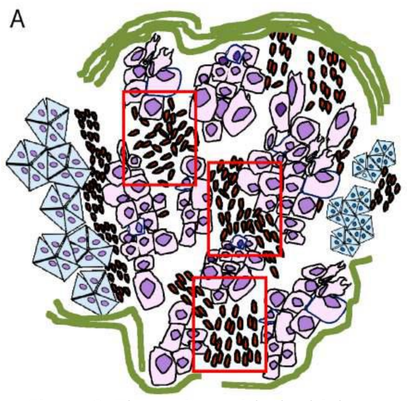


Figure 2. Three sites with the highest infiltration density

(Adapted version from Ino Y. et al. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. // Br. J. Cancer. England, 2013. Vol. 108, № 4. P. 914–923.2013)

Results (EBV-identification)

1. According to the results of assessment of the LMP-1 expression all samples were divided into three groups:

Lauren's cancer type	EBV-positive	EBV-negative	Normal glands*	TOTAL
Intestine type	**7**	**11**	**14**	**32**
Diffuse type	**2**	**21**	**12**	**35**
TOTAL	**9**	**32**	**26**	**67**
p-value	**p = 0,0062**	**p = 0,0055**	**p = 0,2894**	
Differences	**Significant**	**Significant**	**No Significant**	

*EBV-negative gastric cancers with EBV-positive staining of normal glands of the lamina propria

2. Among the EBV-negative cancers with EBV-positive staining of normal glands, signet ring cells (SRC) are more common (p = 0,0425):

Cumulative type	EBV-positive	Normal glands
TOTAL	9	26
SRC+/SRC cancer	2	14

SRC+ = adenocarcinomas with the presence of single signet ring cells
SRC cancer = signet ring cell cancer

3. Among EBV- adenocarcinomas (diffuse type) with EBV+ staining of normal glands, signet ring cells are much more common (p = 0,0024).

Cumulative type	Diffuse type	
	EBV-negative	Normal glands
TOTAL	**9**	**26**
SRC+/SRC cancer	**2**	**14**

SRC+ = adenocarcinomas with the presence of single signet ring cells
SRC cancer = signet ring cell cancer

4. EBV-negative adenocarcinomas are found in the antrum much more often than in the body (p = 0,0292) and much more often than adenocarcinomas with EBV+ staining of normal glands (p = 0,0095).

Localisation	EBV+	EBV-	Normal glands	TOTAL
Antrum	**3**	**10**	**4**	**14**

Conflict of interests

We declare no conflict of interests.

30th European congress of pathology (8-12 September 2018, Bilbao, Spain)

Images (EBV-identification)

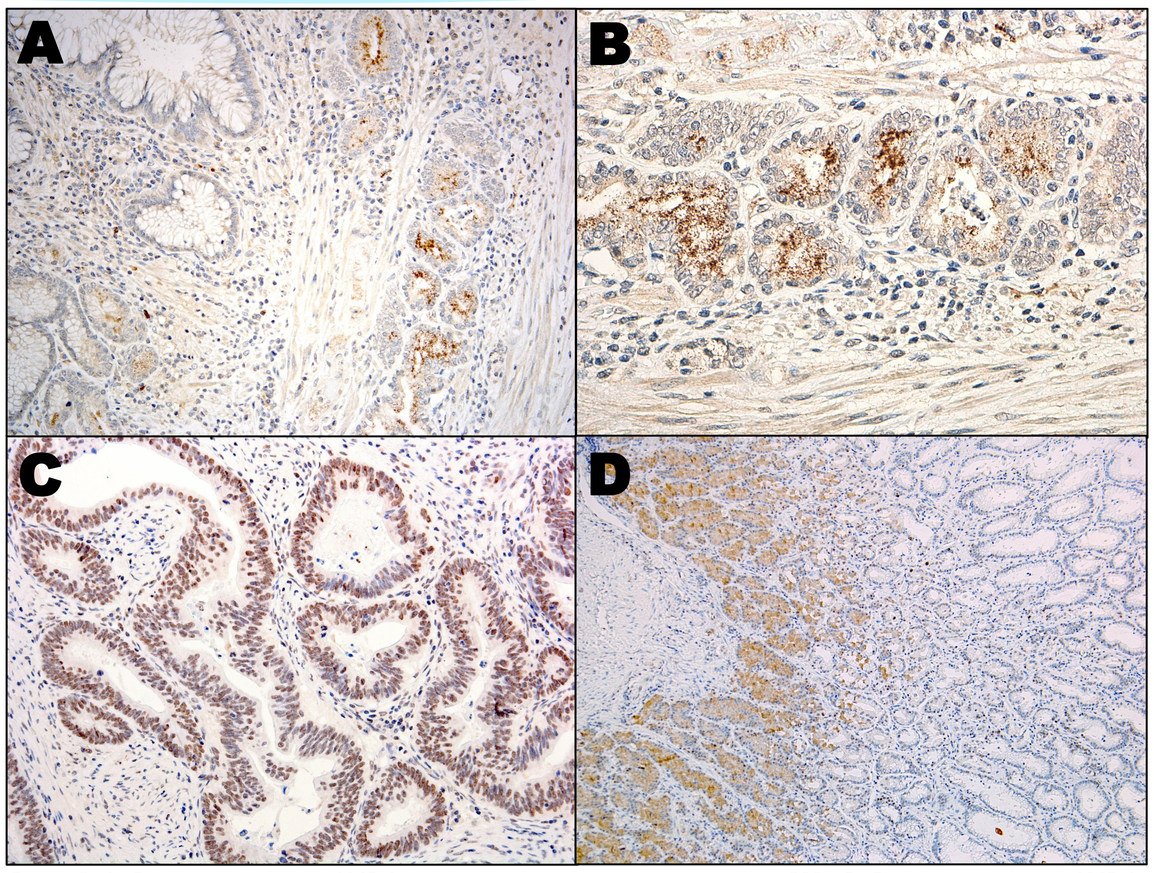


Figure 3. A) Positive cytoplasmic LMP-1-staining in the adenocarcinoma cells (x200); B) Positive cytoplasmic LMP-1-staining in the adenocarcinoma cells (x400); C) False-positive nuclear reaction to LMP-1 in the EBV-negative adenocarcinoma cells (x400); D) Positive cytoplasmic staining of the normal glands in sample with EBV-negative adenocarcinoma (x200, tumor tissue is not represented).

Results (immune microenvironment)

Non-parametric methods were used for the assessment of immune microenvironment: U-Mann-Whitney test Kruskal-Wallis test and Spearman coefficient of linear correlation (significance level p < 0,05).

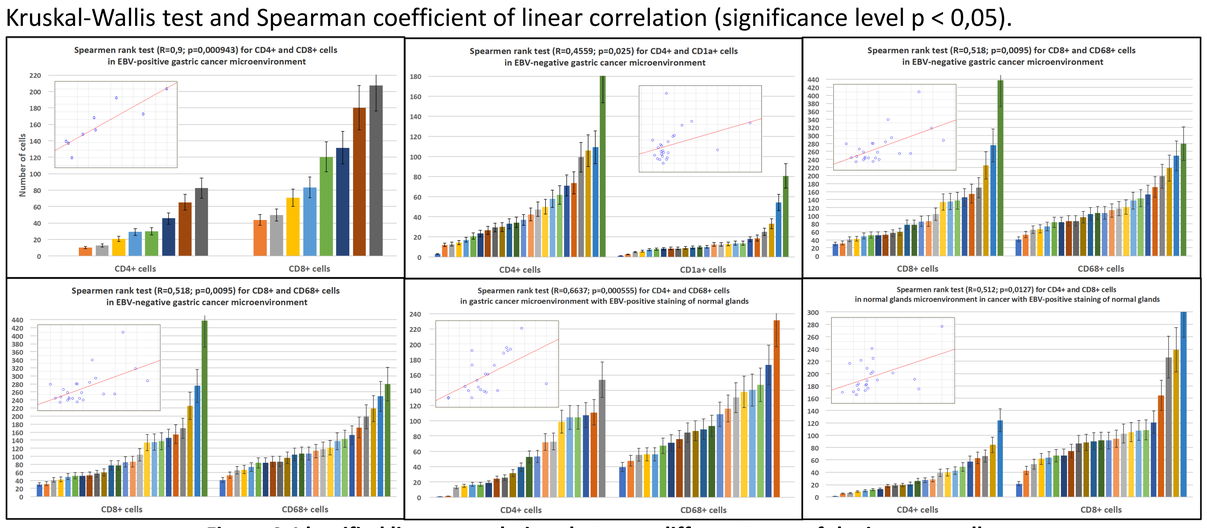
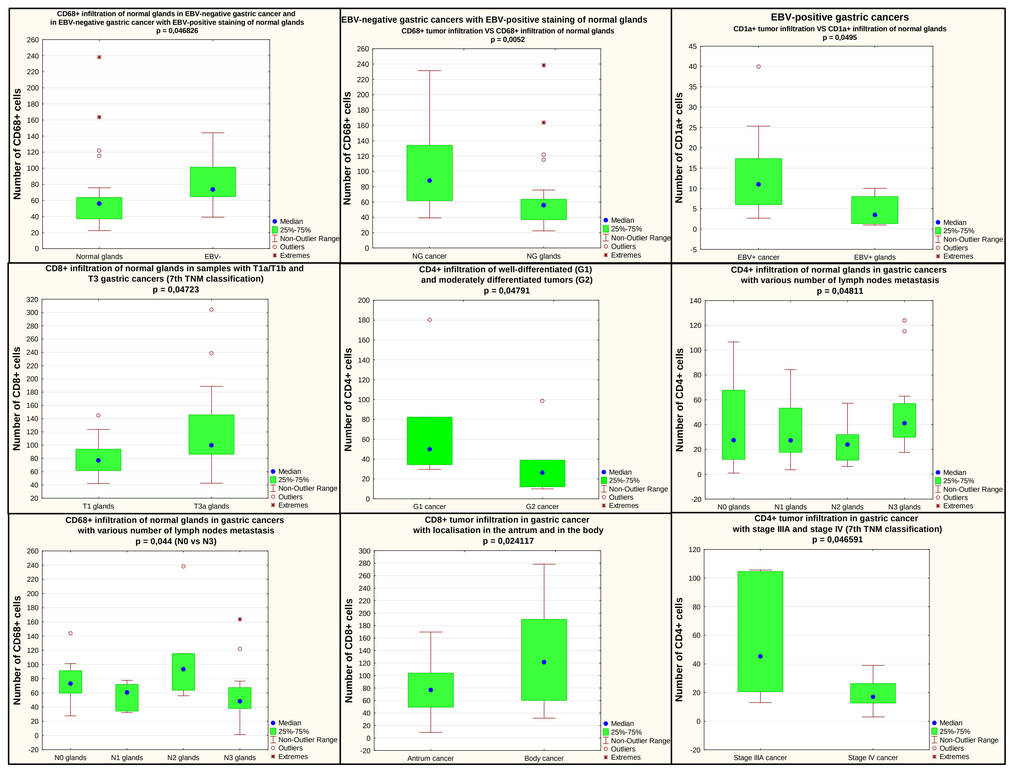


Figure 4. Identified linear correlations between different types of the immune cells.



Conclusions

Immunological properties of tumor tissue and normal glands in gastric adenocarcinomas with EBV-positive normal glands of lamina propria differ significantly from EBV+ and EBV- cancers. We assume that these facts indicate a premalignant process in these glands different from the classical metaplasia-dysplasia-cancer pathway.