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Immunophenotyping of Gastritis, Gastric Ulcer and Gastric Cancer using a Cluster of Differentiation (CD) Antibody Microarray

(Imunofenotip Gastritis, Ulser Gaster dan Kanser Gaster menggunakan Mikroarai Antibodi Kelompok Pembezaan (CD))

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ABSTRACT

One of the factors that contribute to the development of gastric cancer is the host immune response. Extensive immunophenotype of gastric cancer can be identified by using antibody microarray technique that profiles more than 100 cluster of differentiation (CD) antigens in parallel. In this study, we used DotScan™ antibody microarray to profile CD antigen expression in patients with distinct digestive diseases for surface antigen disease-signatures. Patients' blood samples with gastric disorders and healthy controls were taken and processed for leukocytes isolation using Histopaque density gradient centrifugation. Leukocytes were captured onto DotScan™ slides and cell binding densities were analyzed using DotReader™. Different groups of gastric diseases were found to be characterized by differentially expressed distinct CD antigens. Compared to normal healthy controls, 17, two and four highly expressed CD antigens were identified in gastritis, gastric ulcer and gastric cancer patients, respectively. The 17 CD antigens that show differential expression in gastritis were CD15, CD16, CD20, CD23, CD24, CD25, CD28, CD34, CD37, CD77, CD102, CD103, CD122, CD128, CD10, CD183, and CD184. High expression of CD64 and CD69 were found in gastric ulcer, whereas CD52, CD126, CD135, and CD121a were highly expressed in gastric cancer. CD antigens involve in T-cell functions had reduced expression in gastric cancer, while proinflammatory cytokines shows increased expression. These results demonstrate specific immunophenotype of CD antigens in patients with various gastric diseases and identification of differential expressed surface antigens may have clinical significance for diagnostic and therapeutic purposes.

Keywords: Cluster of differentiation antigen; gastric cancer; gastric ulcer; gastritis; immunophenotype

ABSTRAK

Salah satu faktor yang menyumbang kepada perkembangan kanser perut adalah tindak balas imun perumah. Pengenalpastian imunofenotip kanser perut yang meluas dapat dilakukan dengan menggunakan teknik mikroarai antibodi yang memaparkan lebih daripada 100 kelompok antigen pembezaan (CD) secara serentak. Dalam kajian ini, mikroarai antibodi DotScan™ digunakan untuk memprofil pengekspresan antigen CD pada pesakit dengan penyakit pencernaan untuk mengenal pasti penanda antigen permukaan pada pelbagai jenis penyakit pencernaan. Sampel darah pesakit dengan penyakit gaster dan individu kawalan sihat diambil dan diproses untuk pengasingan leukosit menggunakan kaedah emparan kecerunan Histopaque. Leukosit ditangkap ke atas slaid DotScan™ dan kepadatan pengikatan antigen dan antibodi dianalisis menggunakan DotReader™. Keputusan kajian ini menunjukkan kumpulan

penyakit gaster yang berlainan dapat dicirikan oleh ekspresi kelompok antigen CD yang berbeza. Berbanding dengan kawalan normal yang sihat, 17, dua dan empat antigen CD yang diekspres secara berbeza dikenal pasti masing-masing pada pesakit gastritis, ulser gaster dan kanser perut. 17 antigen CD yang menunjukkan perbezaan ekspresi dalam gastritis adalah CD15, CD16, CD20, CD23, CD24, CD25, CD28, CD34, CD37, CD77, CD102, CD103, CD122, CD128, CD10, CD183 dan CD184. Ekspresi CD64 dan CD69 yang tinggi didapati pada penyakit ulser gaster, manakala ekspresi yang tinggi CD52, CD126, CD135 dan CD121a dilihat pada penyakit kanser perut. Antigen CD yang terlibat dalam fungsi sel-T mengalami penurunan ekspresi pada kanser perut, manakala sitokin proinflamasi menunjukkan peningkatan ekspresi. Hasil kajian ini menunjukkan imunofenotip antigen CD adalah khusus pada pesakit dengan pelbagai penyakit gaster dan pengenalpastian antigen permukaan yang diekspres secara berbeza mungkin mempunyai kepentingan klinikal bagi tujuan diagnostik dan rawatan penyakit.

Kata kunci: Gastritis; imunofenotip; kanser perut; kelompok antigen pembezaan; ulser gaster

Introduction

Multiple factors orchestrate gastric cancer carcinogenesis which include *Helicobacter pylori* infection, genetic susceptibility of the host and environmental factors, thus, complex interaction of these factors determine the clinical outcome of the disease. Risk of gastric cancer has been associated with dysregulation of the immune responses and immunosuppression (Bockerstett & DiPaolo 2017; Lee, Hwang & Nam 2014). Diagnosis and prognosis of gastric cancer are poor (Cancer Net Editorial Board 01/2018). By stimulating an efficient immune response to tumour cells, immunotherapy adds a new dimension to the prevention and treatment of gastric cancer and other cancers. The immune system can recognise and kill tumour cells in healthy individuals. This process includes the detection and removal of cancer cells by innate and adaptive immune cells, natural killer cells, T cells, interferons, macrophages and dendritic cells that secrete cytokines (Calder 2007). Suppression or overexpression of these cells lead to aggressive growth of tumour cells. Development of gastric cancer has a long period and involves multistep processes from premalignant lesions to malignant which results from disturbance of the immune responses (Lee, Hwang & Nam 2014). Therefore, identification of specific antigens in premalignant lesions could be utilised to eliminate or prevent tumour cells from ever developing.

A variety of leukocyte molecules involved in the process of immune responses. These molecules were identified by surface components which defined or referred as cluster of differentiation (CD) antigens (Kishimoto et al. 1990). They are involved in innate and acquired immune responses and have different roles, such as interactions between cells, cytokine receptors, cell signaling, ion channels, transporters, enzymes, immunoglobulins or molecules of adhesion (Kishimoto et al. 1990). The expression of CD antigens is different in healthy and disease conditions as well as in different severity of disease states. The extensive immunophenotyping using CD antigens has been reported in diagnosis of several diseases such as leukaemia (Belov et al. 2003, 2001), liver disease (Rahman et al. 2012), colorectal cancer (Zhou et al. 2010), gastric cancer (Sukri et al. 2016) and prediction of HCV recurrence after transplantation (Rahman et al. 2015). In the clinical context, immunophenotype of cells provide opportunities to identify novel disease biomarkers (Sanchez-Carbayo 2011), generating cell protein signature by comparing healthy and disease state and for development of therapeutic antibodies.

CD antibody microarray has advantages compared with other molecular methods. The assay is simpler to perform and can be used in a single assay to screen a large number of antigens (Ellmark et al. 2008). We have previously demonstrated the distribution of CD antigens in gastric cancer cells isolated from gastric mucosal tissues, which play a role in cancer pathogenesis (Sukri et al. 2016). However, our previous study was only limited to profiling CD antigens in gastric mucosal tissue and this technique still requires invasive method in order to retrieve tissue for CD antigen profiling. Thus, profiling CD antigen from peripheral blood presents as an attractive technique for future diagnosis of gastric cancer as well as other gastric diseases by mean of non-invasive method. It is also essential to observe whether highly expressed CD antigens in gastric tissue also highly expressed in peripheral blood cells. No studies have been conducted to extensively profile CD antigens from PBMCs of patients with digestive diseases. Previous studies only use flow cytometry that can detect a few CD antigens at one time. This will be the first study to profile more than 140 CD antigens in peripheral blood of gastric cancer patients and other related diseases by using DotScan™ antibody microarray. The objectives of this study were to determine CD antigen expression profiles for patients with gastric diseases and to look for surface antigen disease-signatures.

Materials and Methods

Patient population

Patients were recruited from Endoscopy Unit, Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Kuala Lumpur, Malaysia from March 2017 to May 2018. The study was approved by the Research Ethics Committee of UKM (UKMPP/111/8/JEP-2017-218). Patients aged more than 18 years old with dyspepsia symptoms who underwent oesophagogastroduodenoscopy (OGDS) were eligible for the study. Inclusion criteria includes patients who were endoscopically diagnosed with gastritis, gastric ulcer and gastric cancer. Exclusion criteria includes patients with NSAID-induced ulcer, patients received proton pump inhibitors or H_2-antagonist two weeks prior OGDS, patients received anticoagulant treatment, patients with chronic kidney disease, diabetes, autoimmune disease and hematological disorders, patients received chemotherapy, and post-gastrectomy patients. Each patient received informed consent and 8 mL of blood was drawn by venipuncture into an EDTA-containing tube. Blood was also collected from volunteer normal healthy individuals without any significant medical history. A total of 27 patients and 7 normal healthy individuals were included in the study with the mean age of 54.21 + 17.94 years old (range: 24 - 81 years old). Patients were endoscopically diagnosed with gastritis (n=14), gastric ulcer (n=9) and gastric cancer (n=4).

Preparation of single-cell suspension from peripheral blood

Isolation of leukocytes was conducted by using density gradient centrifugation. Briefly, blood was carefully overlaid onto the equal volume of Histopaque 1077 (Sigma-Aldrich, United Kingdom) and centrifuged at 400 g for 30 min at room temperature. After centrifugation, mononuclear leukocytes were transferred to a centrifuge tube, washed twice with phosphate buffered solution (PBS) and cell concentration was adjusted to 1 - 4 × 10^6 cells/mL in PBS. Cells with viability more than 80% were proceeded to DotScan™ assay.

DotScan™ CD microarray

CD antibody microarray (DotScan™ slide) were constructed by Medsaic Pty. Ltd. (Eveleigh, NSW, Australia) as described previously (Belov et al. 2001; Sukri et al. 2016). Leukocytes suspensions were transferred onto DotScan™ slide, followed by incubation at room temperature for 30 min. To remove unbound cells, slides were carefully washed twice with PBS. The slides were then fixed in PBS containing 3.7% formaldehyde for 20 min to 1 hour. Subsequently, the slides were washed three times with PBS and the slides were then scanned and analysed using a DotScan™ reader and software (Medsaic Pty. Ltd., NSW, Australia). Dot patterns image of cell binding densities onto

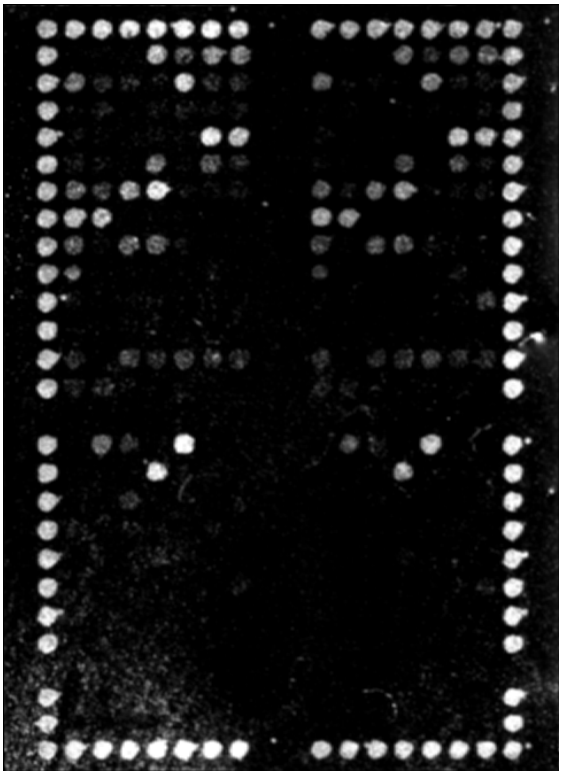

FIGURE 1. Dot patterns of cluster of differentiation (CD) antigens on DotScan™ slides

antibodies were generated as shown in Figure 1. The data obtained were represented as bar graph with CD antigens as x-axis and mean density of cell binding as y-axis (Figure 2). Quantification of dot intensity of each antibody was conducted in duplicate based on 8-bit pixel greyness scale ranging from 0 to 255. Density of complementary surface antigens is proportional to the dot intensity and usually correlates well with findings from flow cytometry (Belov et al. 2003). The general dot pattern is therefore a semiquantitative immunophenotype assayed by the leukocyte population. Data from two housekeeping pan-cellular CD antigens which were CD29 and CD44 showed consistent expression across all samples in this study. Therefore, no further normalization was required. However, any samples with expression of CD29 and CD44 that significantly deviated from the mean were excluded from further analysis.

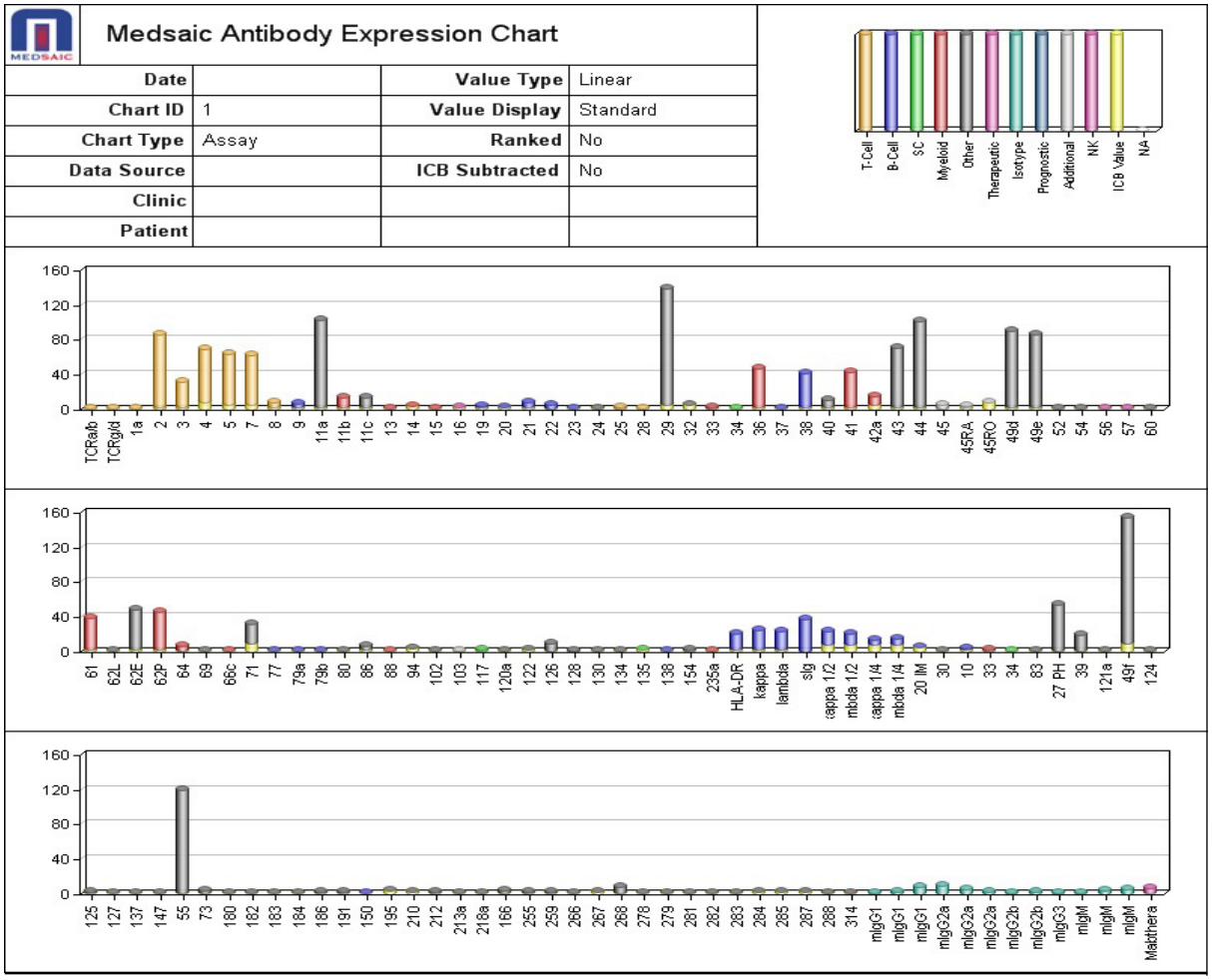

FIGURE 2. The density of cell binding onto each antibody of the CD profiles obtained from the dot patterns

DATA ANALYSIS

Mean fold change of the cell binding densities between healthy and disease groups was calculated to determine a differentially expressed CD antigen. Mann-Whitney with *Exact* test is used to determine the significant difference of the differentially expressed CD antigens in disease compared with normal healthy group. p value less than 0.05 was considered as statistically significant.

RESULTS

In this study, we analyzed the difference in leukocyte CD antigens expression in different group of gastric diseases. Table 1 shows immunophenotyping of CD antigens in different group of diseases compared to normal healthy control. A total of 41 CD antigens were differentially expressed. More differentially CD antigens were observed in patients with gastritis (26 CD antigens) compared to gastric ulcer (14 CD antigens) and gastric cancer (17 CD antigens). Four CD antigens had significantly abundant in gastritis compared with normal healthy (CD57, CD66c, CD128 and CD137, $p < 0.05$). Of the 14 CD antigens that were differentially expressed in gastric ulcer group, 6 CD antigens were repressed (CD3, CD5, CD7, CD9, CD43 and HLA-DR) and 8

CD antigens had high expression (CD33, CD54, CD64, CD69, CD66c, CD138, CD235a and CD30) compared with normal healthy group. As compared to normal healthy, CD3, CD5, CD7 and CD43 were significantly reduced in gastric ulcer ($p < 0.05$), whereas CD235a ($p = 0.030$) and CD30 ($p = 0.006$) were significantly abundant. Of the 17 CD antigens that were differentially expressed in gastric cancer, six CD antigens showed significant differentially expressed. CD3 and CD5 were significantly repressed, whereas CD57, CD126, CD154 and CD124 were significantly abundant in gastric cancer compared with normal healthy.

Table 2 shows the differentially expressed CD antigens distinguishing gastritis, gastric ulcer, and gastric cancer groups. Seventeen distinct CD antigens were differentially expressed in gastritis patients including Lewis x antigen, B cell markers, IL-2, IL-8, receptor for chemokines, ICAM-2, integrin and others. Four CD antigens (CD9, CD64, CD69 and HLA-DR) and five CD antigens (CD8, CD52, CD126, CD135 and CD121a) were distinctively expressed in gastric ulcer and gastric cancer, respectively. CD52 and CD121a were highly expressed in gastric cancers with mean fold change of more than 4 compared to normal healthy.

TABLE 1. Differentially expressed CD antigens in different disease groups compared to normal healthy control

CD Antigen	Type of cell or alternative name	Mean fold change, disease groups vs normal healthy		
		Gast	GU	GC
3	T cell marker	-	-2.06*	-3.64*
5	T cell marker	-	-2.12*	-2.27*
7	T cell marker	-	-2.45*	-2.09
8	T cell marker	-	-	-2.57
9	B cell marker	-	-2.34	-
15	Lewis x	4.15	-	-
16	FcγRIII	3.08	-	-
20	B lymphocyte surface antigen	2.33	-	-
23	B cell marker	2.22	-	-
24	B cell marker	2.91	-	-
25	IL-2 receptor	2.97	-	-
28	Tp44	2.58	-	-
33	Sialic acid-binding Ig	2.12	2.33	-
34	Stem cell marker	2.77	-	-
37	B cell marker	2.10	-	-
43	Sialophrin	-	-2.70*	-4.00
52	Campath-1	-	-	4.07
54	ICAM-1	-	2.38	3.87
57	Human natural killer-1	3.23*	-	2.17*
64	IgG Fc receptor I	-	2.21	-
69	AIM	-	2.09	-
66c	CEACAM6	5.16*	2.47	-
77	Gb3	2.15	-	-
102	ICAM-2	2.18	-	-
103	Intergrin alpha E	2.17	-	-
117	Receptor for stem cell factor	2.00	-	2.02
122	IL-2 receptor β	2.22	-	-
126	IL-6 receptor	-	-	2.20*
128	IL-8 receptor	2.33*	-	-
135	Fms-related tyrosine kinase 3	-	-	2.12
138	Syndecan-1	-	2.17	2.60

154	CD40 ligand	2.31	-	2.55*
235a	Glycophorin A	2.33	2.33*	2.47
HLA-DR		-	-2.09	-
30	TNFRSF8	2.30	2.52*	-
10	Neprilysin	2.92	-	-
121a	IL-1 receptor type 1	-	-	4.96
124	IL-4 receptor	2.37	-	2.65*
137	TNFRSF9	2.16*	-	2.00
183	CXCR3	2.37	-	-
184	CXCR4	2.12	-	-

Gast; gastritis, GU; gastric ulcer, DU; duodenal ulcer, GDU; gastric and duodenal ulcer, GC; gastric cancer; * $p < 0.05$

TABLE 2. Differentially expressed CD antigens distinguishing gastritis, gastric ulcer and gastric cancer groups

CD Antigen	Type of cell or alternative name	Mean fold change (disease groups vs normal healthy)
Gastritis		
CD15	Lewis x	4.15
CD16	FcγRIII	3.08
CD20	B lymphocyte surface antigen	2.33
CD23	B cell marker	2.22
CD24	B cell marker	2.91
CD25	IL-2 receptor	2.97
CD28	Tp44	2.58
CD34	Stem cell marker	2.77
CD37	B cell marker	2.10
CD77	Gb3	2.15
CD102	ICAM-2	2.18
CD103	Intergrin alpha E	2.17
CD122	IL-2 receptor β	2.22
CD128	IL-8 receptor	2.33
CD10	Neprilysin	2.92
CD183	CXCR3	2.37
CD184	CXCR4	2.12
Gastric ulcer		
CD9	B cell marker	-2.34
CD64	IgG Fc receptor 1	2.21
CD69	AIM	2.09
HLA-DR	MHC Class II	-2.09
Gastric cancer		
CD8	T cell marker	-2.57
CD52	Campath-1	4.07
CD126	Interleukin-6 receptor	2.20
CD135	Fms-related tyrosine kinase 3	2.12
CD121a	Interleukin-1 receptor type 1	4.96

Gast; gastritis, GU; gastric ulcer, DU; duodenal ulcer, GDU; gastric and duodenal ulcer, GC; gastric cancer

DISCUSSION

We used the approach of CD antigens immunophenotyping to determine the distribution of CD antigens in gastritis, gastric ulcer, and gastric cancer and to understand if samples of different gastric diseases are associated with different immune process. A strength of this research is that CD antibody profiles can show various classes of diseases and recognize possible signatures of diseases. The significance of this finding in the field of immunotherapy could be useful for the personal care of individuals at risk. Moreover, these data also help to better understand gastric cancer immunopathogenesis. The use of CD antigen expression in various digestive diseases implicates immune responses in disease process. While our research relies on expression of CD antigens by peripheral mononuclear cells, the CD antigen profile for various groups of diseases shows unique signature. The primary goal of this approach is to differentiate the expression of CD antigens between normal and disease groups in order to discover significantly expressed antigens that may play a role in gastric disease prognosis or therapy.

The findings from this study show that different group of gastric diseases can be distinguished by the different of CD antigen profiles. Compared to normal healthy samples, many CD antigens show reduced expression in gastric ulcer and gastric cancer groups. The expression of T cell markers (CD3, CD5 and CD7) were reduced in gastric cancer group as well as gastric ulcer compared to normal healthy. This shows that these cells were crucial for maintenance of normal cells. Pathogenesis of gastric cancer involves different stages of disease from mild to severe. Patients with gastritis might progress to gastric ulcer and ultimately gastric cancer. As gastric ulcer is the precursor for gastric cancer, dysregulation of T cells might be one of the contributing factors that lead to cancer development (Anderson & Rapoport 2018; Oleinika et al. 2013).

The expression of CD antigens in gastric cancer tissues (Sukri et al. 2016) and in peripheral blood of gastric cancer patients (the present study) is compared. CD54 shows increased expression both in cells derived from gastric cancer tissue and peripheral blood. Although the differentially expressed of CD54 in the present study did not reach significant difference, the expression is high than the other groups. The fold change of CD54 in the blood was 3.87 (the present study) compared to 2.79 fold change in the gastric cancer tissue (Sukri et al. 2016). In addition, differentially expressed CD54 was observed in gastric ulcer and gastric cancer with 2.38 and 3.87 fold, respectively, compared with normal healthy group. The findings of differentially expressed CD54 both in the gastric tissue and the blood of gastric cancer patients as well as highly expressed in gastric cancer compared with gastritis and gastric ulcer suggests the potential of this CD54 as a biomarker for gastric carcinogenesis. Further research on CD54 pathogenesis in gastric cancer is needed.

Gastritis group shows a greater number of differentially expressed CD antigens with the expression level of more than 2-fold compared with normal healthy individuals. A high number of unique CD antigens expression in gastritis indicates the complexity of the disease nature as there is several types of gastritis involved (mild, moderate, and severe). In this analysis, we not stratified the gastritis types due to the small number of patients for each gastritis types. The distinctively differentially expressed CD antigens in gastritis include B cell markers (CD20, CD23, CD24 and CD37), Lewis x (CD15), Fc receptor for IgG (CD16), interleukin-2 receptors (CD25 and CD122), type I transmembrane protein (CD28), interleukin-8 receptor (CD128), stem cell marker (CD34), chemokines (CD183 and CD184), Gb3 (CD77), ICAM-2 (CD102), intergrin alpha E (CD103) and neprilysin (CD10).

Expression level of 4 distinct CD antigens (CD9, CD64, CD69, and HLA-DR) in gastric ulcer may be relevant to ulcerogenesis. The motility-related factor CD9 or better known as Tetrasplanin (Miyake et al. 1991) has been shown to play important role in integrin-mediated cell adhesion, motility, growth, differentiation, cell migration and invasion (Levy & Shoham 2005; Powner et al. 2011; Seipold & Saftig 2016). CD9 is amply expressed on the plasma membrane of different myeloid lineage cells such as mast cells (Halova & Draber 2016), basophils (Higginbottom et al. 2000), eosinophils (Fernvik et al. 1995) and macrophages (Huang, Febbraio & Silverstein 2011). Many reports indicate that CD9 is essential in regulation of inflammation and antibody-mediated immune responses (van Spriel 2011), the manufacture of myeloid cells (Oritani et al. 1996) and the expression of cytokines by macrophages (Ha et al. 2005). In the present study, CD9 expression was shown to be repressed in gastric ulcer cases and this may suggest the uncontrolled inflammation occurred that leads to ulcerogenesis. One of the Fc receptors for IgG, CD64 (FcgRI) is constitutively present in macrophages, monocytes and eosinophils and only to a limited degree in resting neutrophils (Li et al. 2013). The upregulated of CD64 expression was associated with the activation of pro-inflammatory macrophages in response to cytokines like tumour necrosis factor-alpha (TNF-) or interferon-gamma(IFN-)

and microbial products. The continuous production of pro-inflammatory cytokines and chemokines promotes chronic inflammation in the gastric mucosa. CD69 is a leukocyte receptor that can be found on small subsets of T and B cells isolated from peripheral lymphoid tissues of healthy subjects (Sánchez-Mateos et al. 1989). CD69 has been shown to reduce the production of multiple proinflammatory mediators via the synthesis of transforming growth factor-β (Sancho et al. 2003). Overexpression of CD64 and CD69 found in ulcer cases in the present study may involve in the development of chronic inflammation in the gastric mucosa. Many forms of cells involved in immune responses, including B-cells, activated T-cells, certain macrophages and dendritic cells, are known to express HLA-DR antigens (Class II histocompatibility antigens) (Daar et al. 1984). HLA-DR is important in antigen recognition and activation of B and T cell response (Thoresby, Berle & Nousiainen 1982; Unanue 1981). HLA-DR expression was increased in gastritis where inflammation occurred, while the expression was reduced in intestinal metaplasia (Wee, Teh & Kang 1992). While ulcer is an intermediate condition between gastritis and intestinal metaplasia, the downregulated of HLA-DR expression found in this study is in agreement with previous reports (Ishii et al. 1992; Wee, Teh & Kang 1992).

A distinct overexpression of CD antigens (CD52, CD126, CD135 and CD121a) were observed in gastric cancer cases. CD52 is a glycoprotein that is present on cell surface of normal and malignant lymphocytes, including T cells and B cells (Valentin et al. 1992; Xia et al. 1993). High expression of CD52 has been reported in various hematologic neoplasms (Albitar et al. 2004; Rodiq et al. 2006) and alemtuzumab (anti-CD52 monoclonal antibody) has been used to treat these diseases (Eketorp et al. 2014). Highly expressed CD52 in gastric cancer cases (4.07 fold) as shown in the present study suggests that CD52 can be used as a potential target for immunotherapy.

IL-6R can be divided into two forms which are membrane-bound protein expressed on hepatocytes and some inflammatory cells, and soluble IL-6R (sIL-6R) that is easily identified in the circulation and at sites of inflammation (Jones, Scheller & Rose-John 2011). Circulation of IL-6 increases significantly during inflammation process. Activation of cells by IL-6 starts with binding of this cytokine to its receptor (IL-6R or known as CD126) that subsequently induces activation of receptor-associated kinases (JAK1, JAK2 and Tyk2) and followed by regulation of STAT1 and STAT3 activity (Heinrich et al. 2003). IL-6 functions as pro-inflammatory cytokine by coordinating leukocyte trafficking and controlling transition from innate immunity to adaptive immunity through leucocyte activation, differentiation and proliferation (Sim 2009). Activity of STAT3 is associated with development of tumour, survival, angiogenesis, and metastatic processes that include cell migration and invasiveness, and reduction of extracellular matrix (Regis et al. 2008; Yu, Pardoll & Jove 2009). Each process is linked and has been shown by the increased expression of IL-6R (CD126) in gastric cancer cases found in the present study.

CD135, also known as FMS-like tyrosine kinase 3 (FLT3) is a class III receptor tyrosine kinase (RTK) and FLT3 RTK activation results in the downstream phosphorylation of secondary mediators that control cell differentiation, proliferation, survival and are necessary for the development of lymphocytes (B cells and T cells) (Lyman 1995). FLT3 is highly expressed in acute myeloid leukemia (Tarlock et al. 2017). In addition, high expression is noticed in a wide degree of hematologic malignancies such as B-cell precursor ALL, some types of T-cell ALL, and chronic myelogenous leukemia in lymphoid blast crisis (Drexler 1996; Tarlock et al. 2017). Differential expression of CD135 found in gastric cancer cases in the present study needs further investigation to elucidate the effects of its high expression in gastric carcinogenesis.

IL-1 is an incredibly strong inflammatory cytokine implicated in countless immune responses covering both innate and adaptive immunity (Dams-Kozlowska et al. 2012). This cytokine binds to primary receptor known as interleukin-1 receptor, type I (IL-1RI or CD121a) (Fields, Günther & Sundberg 2019). Cell signaling induced by IL-1 family is important for regulation of inflammatory response in both innate and adaptive immunity during pathogen infection. However, dysregulation in IL-1 signaling can result in host's auto inflammatory disease. The highly expressed CD121a in gastric cancer cases (4.96 fold) observed in the present study may explain its role in auto-inflammatory effect of the cytokines involve in gastric carcinogenesis.

In conclusion, the ability to discriminate different disease groups based on CD antigens is promising and may reflect surface antigen signatures for various gastric diseases. Current diagnosis of gastric cancer and other gastric diseases is invasive. Therefore, CD antigens identified from this study can be applied as new non-invasive diagnostic biomarkers for discriminating different type of gastric diseases. Downregulation of T cell markers suggests a less effective immune response in patients with gastric ulcer and gastric cancer. This

study does however have limitations. The number of samples are rather small due to stringent criteria was applied for patients' recruitment. This is because CD antigens expression vary in different diseases. Moreover, some of the samples produced low number of cells and viability where the hybridization of the cells onto the slides cannot be proceeded. While the results of the present study are promising, evaluation and validation in a larger number of samples is required to confirm the surface antigen signatures. In addition, further studies of highly expressed CD antigens via cell culture and animal studies are warranted to elucidate its potential in immunotherapy.

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