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Increased number of arginase 1-positive cells in the stroma of carcinomas compared to precursor lesions and nonneoplastic tissues

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ABSTRACT

Arginase 1 (Arg1) is involved in dampening the response of antitumor T lymphocytes. Arg1 expression has been reported in a variety of cancer cell lines and tumor-associated myeloid-derived cells. However, its examination *in situ* in tumor microenvironment is poorly investigated. We examined the Arg1-positive cells in tumor microenvironment of gastric carcinomas (GCs), colorectal carcinomas (CRCs) and prostate carcinomas (PCs), and analyzed their clinicopathological significance. Immunohistochemical staining for Arg1 was done in 60 GCs, 38 gastric adenomas, 40 CRCs, 10 colonic adenomas, 36 PCs, and 15 benign prostatic hyperplasia (BPH). Arg1 expression was predominantly localized in tumor microenvironment and the stroma of nonneoplastic tissues. Cells with Arg1 expression were mostly leukocytes, morphologically resembling polymorphonuclear neutrophils, and showed CD15 expression. Arg1 expression was focally expressed in cancer cells of 6 PCs, but not in those of GCs and CRCs. Arg1-positive cells were significantly more infiltrated in tumors than adenomas and nonneoplastic tissues, such as BPH, intestinal metaplasia and adjacent tissues. There were no significant findings between them and clinicopathological parameters, except for the relationship to gender and tumor differentiation in CRCs. These findings suggest that Arg1-positive cells in tumor microenvironment is involved in the occurrence of GCs, CRCs, and PCs. More expansive studies are necessary to better elucidate their clinicopathological significance in carcinomas.

1. Introduction

There is a dynamic immunological process between the immune system and tumors [1]. The host immunosurveillance network removes tumor cells during the elimination phase. However, less immunogenic tumor cells survive during this period and progressively develop immunological mechanisms to escape from the host immune system. Tumor immunosuppressive microenvironment contributes to them. Briefly, this is accomplished by the combined action of anti-inflammatory mediators, inhibitory molecules, and immunoregulatory cells. The latter includes regulatory T cells (Treg) and myeloid-derived cells, such as macrophages, dendritic cells, granulocytes, and myeloid-derived suppressor cells (MDSC) [2,3].

Both tumor cells and tumor-associated myeloid-derived cells overexpress and secrete arginase 1 (Arg1) [2]. It converts extracellular L-arginine to ornithine and urea, resulting in the depletion of extracellular L-arginine [4]. Extracellular arginine plays an important role in the activation and proliferation of T lymphocytes via the upregulation of CD3ζ, suggesting that Arg1 is involved in dampening the response of antitumor T lymphocytes [5,6]. Arginase expression has been reported in a variety of cancer cell lines, such as colorectal cancer, nonsmall cell lung cancer, renal cell cancer, breast cancer, prostate cancer and gastric cancer [2]. Arginase in prostate cancer functionally suppressed tumor-infiltrating lymphocytes [7]. Arg1 was also expressed in tumor- associated myeloid-derived cells, which facilitates cancer immune evasion [2,3]. Therefore, Arg1 blockade by CB-1158 or the disruption of signal transducer and activator of transcription (STAT), downstream of Arg1, inhibited the immunosuppressive activity of tumor-associated myeloid-derived cells in human prostate cancer and murine cancer models [3,8].

To the best of our knowledge, examination *in situ* of Arg1 expression in tumor microenvironment of human cancers is poorly investigated. As far as we know, two previous studies have shown this by im-

Abbreviations: Arg1, arginase 1; T reg, regulatory T cells; GCs, gastric carcinomas; CRCs, colorectal carcinomas; PCs, prostate carcinomas; BPH, benign prostatic hyperplasia; MDSC, myeloid-derived suppressor cells; STAT, signal transducer and activator of transcription; M, monocytic; G, granulocytic

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Table 1
Characteristics of current subjects.

Group		Number	Age	Gender (Male:Female)
Stomach				
	Carcinoma	60	41 ~ 86	39:21
	Adenoma	38	53 ~ 82	19:19
	Adjacent tissue			
	Intestinal metaplasia	31	53 ~ 83	21:10
	Normal	26	45 ~ 81	12:14
Colon				
	Carcinoma	40	54 ~ 86	24:16
	Adenoma	10	43 ~ 72	5:5
	Adjancet tissue	40	54 ~ 86	24:16
Prostate				
	Carcinoma	36	59 ~ 78	
	Adjacent tissue	36	59 ~ 78	
	BPH	15	62 ~ 79	

munohistochemistry. In non-small cell lung cancer, Arg1 was expressed in tumor infiltrating neutrophils within the tumor, where its intensity was reduced compared with the neutrophils in the peritumoral or intravascular area, suggesting that Arg1 was released in tumor microenvironment [9]. In mycosis fungoides, Arg1-positive cells were regarded as MDSC, and their numbers were increased in the early stages, suggesting that they were involved in the progression of mycosis fungoides at early stages [10]. The current study examined Arg1-positive cells in the tumor microenvironment of gastric carcinomas (GCs), colorectal carcinomas (CRCs), and prostate carcinomas (PCs) by immunohistochemical staining, and analyzed their clinicopathological significance.

2. Materials and methods

2.1. Patients, tissue samples and clinicopathological analysis

There were 60 patients with GCs, 38 with gastric adenomas, 40 with CRCs, 10 with colonic adenomas, 36 with PCs, and 15 with benign prostatic hyperplasia (BPH). These tissues were obtained through curative surgery, endoscopic dissection, and transurethral resection at Dongguk University Gyeongju Hospital, between January 2009 and December 2015. All cancer patients had not undergone preoperative chemotherapy. Intestinal metaplasia and adjacent nonneoplastic tissues were used in the tissues of GCs, CRCs, and PCs. The characteristics of the study subjects are summarized in Table 1. A pathologist who was unaware of the immunohistochemical results for Arg1 reviewed the clinicopathological features using hematoxylin and eosin stained sections and medical records. Gastric and colonic adenomas were diagnosed when there were cytological and structural atypia without definite tumor invasion. This study was approved by the Institutional Review Board of Dongguk University Gyeongju Hospital.

2.2. Immunohistochemistry

Tissue preparation and immunohistochemical staining were performed as previously described [11]. In brief, tissue sections with a thickness of 4 μm were prepared and spread on poly-l-lysine-coated slides. The paraffin sections were then immersed in three changes of xylene and hydrated using a graded series of alcohol solutions. Antigen retrieval was performed routinely by immersing the sections in a 0.01 M citrate buffer (pH 6.0) in an autoclave for 15 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 15 min, after which the sections were incubated with anti-Arg1 antibody (1:1000, Gene Tex, Irvine, CA, USA) and anti-CD15 antibody (1:250, Dako, Santa Barbara, CA, USA) at room temperature for 2 h. Immunohistochemical staining was performed using an EnVision kit (Dako) and the color was developed using 3, 3-diaminobenzidine tetrahydrochloride (Zymed Laboratories, Inc., South San Francisco, CA, USA) as a chromogen. The sections were counterstained with Meyer's hematoxylin for 3 min, then mounted. Rabbit IgG isotype was used as a negative control. Arg1 positive cells were estimated in tumor microenvironment and the stroma of nonneoplastic tissues such as adjacent tissues, intestinal metaplasia and BPH. They were counted in 20 randomly selected high power fields and averaged for statistical analysis.

2.3. Statistical analysis

One-way ANOVA, t-test, and Pearson correlation coefficient were used to identify any differences among the groups. All analyses were performed using SPSS 23.0 statistical software (SPSS Inc, Chicago, IL, USA). A $P < 0.05$ was considered to indicate statistical significance. All data were expressed as the mean ± standard error.

3. Results

Arg1 expression was predominantly localized in tumor microenvironment and the stroma of nonneoplastic tissues. As shown in Fig. 1, the cells with Arg1 expression were mostly leukocytes, morphologically resembling polymorphonuclear neutrophils, and showed positive signals for CD15. Moreover, as shown in Fig. 1, Arg1 expression was focally expressed in cancer cells of 6 PCs, but not found in the cancer cells of GCs and CRCs.

In the case of GCs, including gastric adenomas and nonneoplastic tissues, such as intestinal metaplasia and adjacent tissues, the number of Arg1-positive cells was progressively increased from nonneoplastic tissues to adenomas and GC, as shown in Fig. 2. Their numbers were 33.5 ± 5.3 in GCs, 14.8 ± 2.6 in adenomas and 4.4 ± 0.8 in

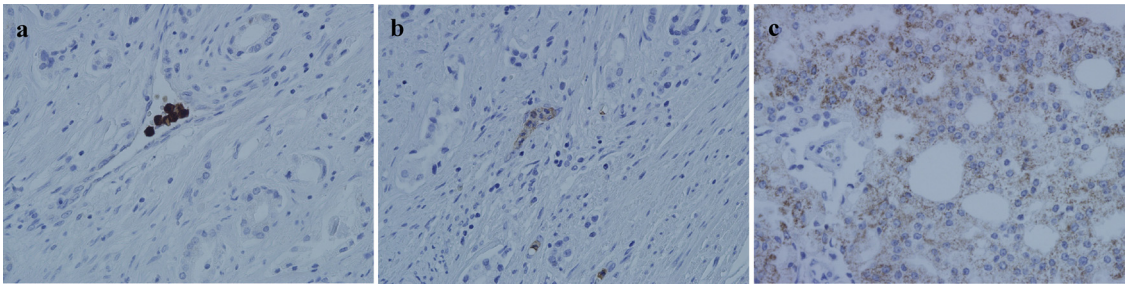

Fig. 1. Immunohistochemical staining of Arg1 (a and c) and CD15 (b) in prostate carcinoma (400×). Arg1 positive cells are mostly leukocytes that morphologically resemble polymorphonuclear neutrophils and also show positive signals for CD15 (a and b). Arg1 expression is focally expressed in cancer cells (c).