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ORIGINAL ARTICLE

PLA2G3 phospholipase expression patterns in colon adenocarcinoma

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Summary

Purpose: *Group III phospholipase A_2 (PLA2G3) is a main member of proteins that are involved in functions such as fatty acid metabolism and oxidative stress response. At the biochemical level catalyzes the calcium-dependent hydrolysis of the 2-acyl groups in 3-sn-phosphoglycerides. Our aim was to investigate the expression of Prdx-6 in colon adenocarcinoma (CA).*

Methods: *A series of 30 formalin-fixed, paraffin-embedded primary CAs tissue sections were used and analyzed. Immunohistochemistry was performed using an anti-PLA2G3 antibody. Digital image analysis was also implemented for evaluating objectively the protein expression levels on the corresponding stained cells.*

Results: *PLA2G3 protein overexpression (increased immunostaining levels) was observed in 23/30 (76.6%) cases, whereas 07/30 (23.40%) CA tissues demonstrated low protein levels, respectively. PLA2G3 overall expression was strongly associated with the grade and tumor localization of the examined tumors (p=0.001, p=0.027, respectively), whereas other statistical significances were not assessed (inflammatory infiltration: p=0.846; stage: p=0.755; tumor diameter: p=0.300; ulceration: p=0.872; gender: p=0.902).*

Conclusions: *PLA2G3 overexpression is observed in a significant subset of CAs associated with aggressive biological behavior (neoplasm dedifferentiation) and also a kind of selectivity in localization of the malignant substrate. PLA2G3 seems to be an important enzyme for endogenous cell response to increased oxidative stress and fatty acid metabolic imbalances in CAs. For these reasons, it should be considered as a potential biomarker and therapeutic target in these malignancies.*

Key words: *carcinoma, colon, PLA2G3, immunohistochemistry, oxidative stress, metabolism*

Introduction

Colon adenocarcinoma (CA) demonstrates a well analyzed model of genomic instability in solid malignancies [1]. Extensive molecular analyses have shown that two main mechanisms of genetic deregulation are involved in the malignant transformation of normal colon glandular epithelia: chromosomal and specific gene imbalances, respectively [2]. Gross chromosomal instability, as the result of chromosomal polysomy/aneuploidy, is detected in the majority (~85%) of CA, whereas the rest demonstrate chromosomal stability (diploid predominantly patterns) [3]. Additionally, a

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subset of patients with CA is characterized by a complex genetic signature and molecular heterogeneity due to a combination of these mechanisms [4,5].

Focused on biochemical and metabolic deregulations that occur in the intra-cellular microenvironment during the carcinogenetic process, elevated oxidative stress is a crucial event [6]. Interestingly, the role of aberrant H_2O_2 inta-cellular concentration is critical, leading to enhanced oxidative stress, motivating also redox signaling pathways [7,8]. Furthermore, abnormal fatty acid and lysophospholipids metabolism due to specific genes' alterations is related with increased risk for epithelial neoplastic transformation, as experimental studies showed regarding primary melanoma [9]. Concerning molecules that regulate these metabolic procedures, secretory phospholipases are most important. These enzymes regulate biochemical and metabolic pathways in the intra-cellular micro-environment by catalytically hydrolyzing fatty acids in order arachidonic and lysophosphatidic acids to be released. This activity is normally detected in a variety of cells and functions of epididymal sperm maturation and fertility [10]. Among them, secretory phospholipase A_2 (sPLA2) gene (PLA2G10 gene band: 16p13.12) encodes for the corresponding protein implicated in biochemical reactions including insulin metabolism, atherosclerosis and response to inflammation. Focused on its last role, sPLA2 acts as a regulator of proximal and also distal reactions in the inflammatory pathways [11]. Besides sPLA2, Group III phospholipase A_2 (PLA2G3) -encoded by the corresponding gene (22q12.2) - is under investigation in carcinomas, including CA rise and progression [12]. In the current study we explored the role of PLA2G3 protein expression patterns in a series of CAs.

Methods

Study group

For the purposes of our study, 30 archival, for¬malin-fixed and paraffin-embedded tissue specimens of histologically confirmed primary CAs were used. The hospital ethics committee consented to the use of these tissues in the Department of Pathology, Halkida General Hospital, Greece, for research purposes, according to World Medical Association Declaration of Helsinki guidelines.

Table 1. Clinicopathological parameters and total PLA2G3 expression results

Clinicopathological parameters		*PLA2G3*		*p value*
CAs (n=30)	*n (%)*	*OE*	*LE*	
		23/30	*07/30*	
		(76.6%)	*(23.4%)*	
Gender				0.902
Male	15/30 (50)	12/30 (40)	3/30 (9.9)	
Female	15/30 (50)	11/30 (36.6)	4/30 (13.3)	
Grade				0.001
I	1/30 (3.3)	0/30 (0)	1/30 (3.3)	
II	22/30 (73.3)	16/30 (33)	6/30 (19.8)	
III	7/30 (23.3)	7/30 (23.3)	0/30 (0)	
Stage				0.755
I	3/30 (10)	2/30 (6.6)	1/30 (3.3)	
II	13/30 (43.3)	9/30 (30)	4/30 (13.3)	
III	10/30 (33.3)	9/30 (30)	1/30 (3.3)	
IV	4/30 (13.3)	3/30 (9.9)	1/30 (3.3)	
Anatomical location				0.027
Sigmoid-Rectum	21/30 (70)	15/30 (50)	6/30 (19.8)	
Ascending-Caecum	9/30 (30)	8/30 (26.4.8)	1/30 (3.3)	
Inflammatory infiltration				0.846
Yes	25/30 (83.5)	19/30 (62.7)	6/30 (19.8)	
No	5/30 (16.7)	4/30 (13.2)	1/30 (3.3)	
Ulceration				0.872
Yes	5/30 (16.5)	4/30 (13.2)	1/30 (3.3)	
No	25/30 (83.5)	19/30 (62.7)	6/30 (19.8)	

CAs: colon adenocarcinomas, PLA2G3: Group III phospholipase A_2, OE: overexpression (high/moderate expression) staining intensity values ≤132at stained cells, LE: low expression staining intensity values >142 at ≤161 at stained cells

The tissue samples were fixed in 10% neutral-buffered formalin. Hematoxylin and eosin (H&E)-stained slides of the cor¬responding samples were reviewed for establishing the corresponding histopathological diagnoses. All lesions were classified according to the histological typing and staging criteria of World Health Organization (WHO) digestive system tumors classification [13]. Clinicopathological data of the examined cases are demonstrated in Table 1.

Antibodies and immunohistochemistry assay (IHC)

We selected and applied the rabbit polynoclonal anti-PLA2G3 antibody (ab62197, Abcam, USA; dilution 2.5μg/ml). IHC protocol for the antigen detection was carried out on a 3 μm-thick paraffin sections of the current blocks. Tissue sections initially deparaf¬finized in xylene and rehydrated via graded ethanol – were immunostained according to the EN Vision+ (DAKO, Denmark) assay using an automated staining system (I 6000 - Biogenex, CA, USA) and according to the corresponding antibodies manufacturer's instruc¬tions. This specific assay is based on a soluble, dextran-polymer system preventing endogenous biotin reaction and increasing the quality of the stained slides. Briefly, the sections, after peroxidase blocking, were incubated with primary antibody for 30 min at room temperature and then incubated with Horseradish peroxidase labeled polymer-HRP LP for 30 min. A wash with TBS was performed. The antigen-antibody reaction was visualized using 3-3, diaminobenzidine tetrahydrocloride (DAB) as a chromogen substrate (8 min at room temperature). Finally, the tissue sections were slightly counterstained with hematoxylin for 30 sec, dehydrated and mounted. For negative control slides, the primary antibodies were omitted. Focal or diffuse cytoplasmic/membranous staining pattern was considered to be acceptable for the marker. Normal colon epithelia tissue sections demonstrating PLA2G3 expression were used as positive markers for its immunostaining pattern, according to antibody manufacturer's instructions (Figure 1a).

Digital image analysis assay (DIA)

PLA2G3 protein expression levels were evaluated quantitatively by measuring the corresponding staining intensity levels (densitometry analysis) in the stained malignant cells. We performed DIA using a semi-automated system (hardware: Microscope CX-31, Olympus, Melville, NY, USA, Digital camera, Sony, Tokyo, Jp; Windows XP/NIS-Elements Software AR v3.0, Nikon Corp, Tokyo, Japan). Areas of interest per tissue section were identified (5 optical fields at ×400 magnification) and filed in a digital database as snapshots. Measurements were performed by implementing a specific macro (cytoplasmic and membrane expression for tumor cells, according to manufacturer's datasheet). Based on an algorithm, normal tissue sections (control) were measured independently and compared to the corresponding values in malignant tissue sections. A broad spectrum of continuous grey scale values (0-255) at the RedGreenBlue (RGB) analysis was available for discriminating different protein expression levels (Figure 1b). Immunostaining intensity values decreasing to 0 represent a progressive overexpression of the marker, whereas values increasing to 255 show a progressive loss of its staining intensity. Total results and DIA values are demonstrated in Table 1.

Statistics

For statistical analyses, descriptive and inferential techniques were applied. Quantitative variables were presented as mean ± standard deviation, while the qualitative variables were presented in frequency tables. To evaluate the relationship between qualitative and quantitative variables, due to the small number of subjects in each group, the non-parametric Mann-Whitney U test and Kruskall-Wallis test were applied. To evaluate the relationship between independent qualitative variables, where appropriate, the control x^2 for linear trend and the control of Fisher were applied. Statistical significance (p) was evaluated in pairs and differences < 0.05 were considered statistically significant. Total IHC results and differences (p values) are described in Table 1.

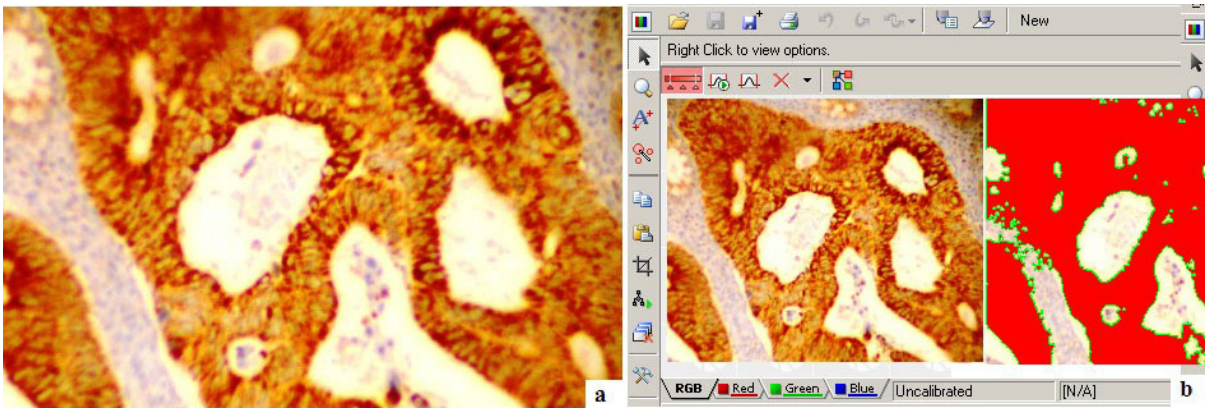

Figure 1. Group III phospholipase A_2 (PLA2G3) protein expression in a colon adenocarcinoma tissue section. **a:** High expression staining intensity level (diffuse cytoplasmic/membranous staining pattern; anti- PLA2G3 antibody, DAB stain, original magnification: 400×). **b:** PLA2G3 protein digital image expression analysis in colon adenocarcinoma. Progressive measurements in three stages: red/green areas represent different levels of protein expression as staining intensity values (diffuse cytoplasmic staining pattern; anti- PLA2G3, DAB stain, original magnification: 400×).

Results

According to digital expression analysis, the examined immunostained CA tissue sections demonstrated different expression levels of PLA2G3 marker. PLA2G3 protein overexpression (increased immunostaining levels: moderate and high) was detected in 23/30 (76.6%) cases, whereas 07/30 (23.40%) CA tissues demonstrated low protein levels. PLA2G3 overall expression was strongly associated with the grade and tumor localization of the examined tumors (p=0.001, p=0.027, respectively). Interestingly, a progressive overexpression was observed from stage I/II to stage III cases. Concerning the influence of PLA2G3 expression in the other clinico-pathological parameters, statistical significances were not assessed (inflammatory infiltration: p=0.846; stage: p=0.755; tumor diameter: p=0.300; ulceration: p=0.872; gender: p=0.902).

Discussion

PLA2G3 is a main member of proteins that are involved in functions such as fatty acid metabolism and oxidative stress response. At the biochemical level it catalyzes the calcium-dependent hydrolysis of the 2-acyl groups in 3-sn-phosphoglycerides [14]. Interestingly, an extensive cDNA-based cloning and coding analysis for human group III sPLA(2) has identified structural and functional similarities in mammalian secretions and venom released by invertebrates -such as bees and scorpions [15]. Furthermore, the study group revealed that PLA2G3 is a Ca (2+)-dependent enzyme showing an 11-fold preference for phosphatidylglycerol over phosphatidylcholine and optimal activity at pH 8. It is also known that PLA2G3 and fibroblast prostaglandin synthase promote mast cell maturation and function by activating a mast cell-fibroblast - dependent paracrine axis [16]. A lipid-driven PLA2G3-L-PGDS-DP1 loop that drives mast cell maturation has been also reported as an alternative mast cell maturation mechanism correlated with anaphylaxis phenomenon [17]. For this reason, PLA2G3 is involved in various pathophysiological events including allergic type reactions [18]. Concerning the PLA2G3 aberrant expression in non-neoplastic diseases, there are important published data that show a strong relation between PLA2G3 gene silencing and Alzheimer's disease as a result of neuronal damage induced by oxidative stress at free radical-generating xanthine/xanthine oxidase (X-XOD) system [19].

In the current study we explored the role of PLA2G3 expression in CA. We observed a significant impact of the molecule in the grade of the examined tumors and selectivity in localization of the malignant substrate. PLA2G3 aberrant expression has been already found to be correlated with survival rates and metastatic potential modifying also chemo-sensitization in solid malignancies like ovarian cancer [20]. Focused on PLA2G3 deregulation in colon neoplastic and non-neoplastic lesions, lipidomics-based analyses reported important pro-inflammatory/pro-tumorigenic lysophosholipids production combined with upregulation of colon-protective fatty acids and oxygenated metabolites [21] Aberrant secreted sPLA2-III expression were detected in inflammatory (colitis) and also in CAs, revealing a progressive mechanism of fatty acid metabolism alterations. Another study analyzed a series of sporadic CAs by implementing a combination of quantitative PCR and immunohistochemistry. Overexpression of PLA2G3 protein in CA tissues was correlated to increased metastatic potential (lymph node-positivity) demonstrating differences regarding the anatomic localization [22]. Interestingly, genetic variability analyses of fatty acid metabolism-related genes reveal significant results regarding specific genetic signatures among patients with CAs. A study group identified that hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD), phospholipase A2 group VI (PLA2G6) and transient receptor potential vanilloid 3 were related with a high risk for CA, whereas prostaglandin E receptor 2 (PTGER2) was associated with lower risk. Similarly, HPGD AGGAG and PLA2G3 CT haplotypes are associated with a high risk for CA [23]. In addition, single nucleotide polymorphisms (SNPs) in fatty acid metabolism genes such as SNP rs12299484 are correlated to high risk for colon carcinogenesis [24].

In conclusion, PLA2G3 overexpression is observed in a significant subset of CAs associated with aggressive biological behavior (neoplasm dedifferentiation) and also a kind of selectivity in localization of the malignant substrate. PLA2G3 seems to be an important enzyme for endogenous cell response to increased oxidative stress and fatty acid metabolic imbalances in CAs. For these reasons, it should be considered a potential biomarker and therapeutic target in these malignancies. Further protein and molecular studies based on the molecule's deregulation mechanisms in cancerous and non-cancerous colon epithelia could reveal unexplored intra-cellular pathways regarding cell responses to oxidative damages in a variety of carcinomas combined with other critical enzymes, such as Peroxiredoxin 6 (PRDX6) [25].

Conflict of interests

The authors declare no conflict of interests.

References

1. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61: 759-67.
2. Salk JJ, Fox EJ, Loeb LA. Mutational heterogeneity in human cancers: origin and consequences. Ann Rev Pathol 2010; 5: 51–75.
3. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell 2011; 144: 646-74.
4. Tang R, Changchien CR, Wu MC et al. Colorectal cancer without high microsatellite instability and chromosomal instability-an alternative genetic pathway to human colorectal cancer. Carcinogenesis 2004; 25:841-6.
5. Koketsu S, Watanabe T, Tada T, Kanazawa T, Ueda E, Nagawa H. Sporadic colorectal cancer in elderly people. Hepatogastroenterology 2003; 50:1749-52.
6. Sies H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: oxidative eustress. Redox Biol 2017; 11:613-9.
7. Sies H. Role of metabolic H2O2 generation: redox signaling and oxidative stress. J Biol Chem 2014; 289: 8735-41.
8. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Free Radic Biol Med 2012; 53: 1522-30.
9. Gorlov I, Orlow I, Ringelberg C et al. Identification of gene expression levels in primary melanoma associated with clinically meaningful characteristics. Melanoma Res 2018; 28:380-9.
10. Sato H, Taketomi Y, Isogai Y et al. Group III secreted phospholipase A_2 regulates epididymal sperm maturation and fertility in mice. J Clin Invests 2010; 120:1400-14.
11. Sato H, Taketomi Y, Isogai Y et al. Group III secreted phospholipase A_2 transgenic mice spontaneously develop inflammation. Biochem J 2009; 421:17-27.
12. Murakami M, Masuda S, Shimbara S, Ishikawa Y, Ishii T, Kudo I. Cellular distribution, post-translational modification, and tumorigenic potential of human group III secreted phospholipase A(2). J Biol Chem 2005; 280:24987-98.
13. Nagtegaal ID, Odze RD, Klimstra D et al. The 2019 WHO classification of tumours of the digestive system. Histopathology 2020; 76:182-8.
14. Zhang S, Gong W, Han Z, Liu Y, Li C. Insight into Shared Properties and Differential Dynamics and Specificity of Secretory Phospholipase A_2 Family Members. J Phys Chem B 2021; 125: 3353-63.
15. Valentin E, Ghomashchi F, Gelb MH, Lazdunski M, Lambeau G. Novel human secreted phospholipase A(2) with homology to the group III bee venom enzyme. J Biol Chem 2000; 275:7492-6.
16. Starkl P, Marichal T, Galli SJ. PLA2G3 promotes mast cell maturation and function. Nat Immunol 2013;14:527-9.
17. Taketomi Y, Ueno N, Kojima T et al. Mast cell maturation is driven via a group III phospholipase A_2-prostaglandin D2-DP1 receptor paracrine axis. Nat Immunol 2013; 14:554-63.
18. Murakami M, Taketomi Y. Secreted phospholipase A_2 and mast cells. Allergol Int 2015; 64:4-10.
19. Martínez-García A, Sastre I, Recuero M et al. PLA2G3, a gene involved in oxidative stress induced death, is associated with Alzheimer's disease. J Alzheimers Dis 2010; 22:1181-7.
20. Ray U, Roy D, Jin L et al. Group III phospholipase A_2 downregulation attenuated survival and metastasis in ovarian cancer and promotes chemo-sensitization. J Exp Clin Cancer Res 2021; 40:182.
21. Murase R, Taketomi Y, Miki Y et al. Group III phospholipase A_2 promotes colitis and colorectal cancer Sci Rep 2017; 7:12261-67.
22. Mounier CM, Wendum D, Greenspan E, Fléjou JF, Rosenberg DW, Lambeau G. Distinct expression pattern of the full set of secreted phospholipases A_2 in human colorectal adenocarcinomas: sPLA2-III as a biomarker candidate. Br J Cancer 2008; 98:587-95.
23. Hoeft B, Linseisen J, Beckmann L et al. Polymorphisms in fatty-acid-metabolism-related genes are associated with colorectal cancer risk. Carcinogenesis 2010; 31:466-72.
24. Crous-Bou M, Rennert G et al. Genetic polymorphisms in fatty acid metabolism genes and colorectal cancer. Mutagenesis 2012;27:169-76.
25. Falidas E, Kitsiouli E, Tsounis D et al. Impact of Peroxiredoxin-6 expression in colon adenocarcinoma JBUON 2021; 26:1893-97.