Expression of pepsinogen A (PGA/PG I) in breast cancer and non-cancer tissues and its relationship with clinicopathological parameters of breast cancer

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Research Article

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Abstract

Background

Human pepsinogens (PGs) are inactive pro-enzymes for the specific digestive enzyme—pepsin originating from the gastric mucosa, Pepsinogens A (PGA) is one of pepsinogens family members. In the past, relevant studies in PGA mainly focused on gastric diseases, but there were few reports outside the stomach. Further understanding of the relationship between PGA and extra-gastric diseases, especially cancer, is helpful to fully understand the role and function of PGA.

Methods

Total of 362 patients with different breast disease after surgery were registered in the study. Immunohistochemical staining was used for PGA expression. GEO, and a series of software packages based on R language were used for further validation as well as function analysis of PGA in breast cancer.

Results

There were significant statistical differences of PGA expression between breast cancer and non-cancer tissues including different benign breast diseases. The results of correlation between PGA expression and clinicopathological parameters of breast cancer showed that there was no significant correlation between PGA expression and general clinicopathological parameters except molecular classification of breast cancer. Analysis with GEO showed that higher PGA expression may indicate a poor prognosis of breast cancer. GO and KEGG analysis showed that PGA may be involved in PPAR signaling pathway and AMPK signaling pathway regulation mechanisms.

Conclusions

The expression of PGA in breast cancer was significantly higher than that of non-cancer tissues, and it was related to molecular classification of breast cancer. the prognosis of patients with higher PGA mRNA expression was poorer. PGA may function through interactions with PPAR signaling pathway and AMPK signaling pathway in breast cancer.

Introduction

Human pepsinogens (PGs) are inactive precursors of pepsin, which is an specific digestive enzyme. Generally, PGs can be classified biochemically and immunochemically into pepsinogen A/I (PGA/PGI) and pepsinogen C/II (PGC/PGII). Mainly synthesized and secreted by the chief cells and the mucous neck cells of the gastric mucosa, PGs could be converted into pepsin under acidic conditions, and thus

participate in the process of digestion[1]. In addition to the stomach, recently researchers have also detected the expression of pepsinogens in some non-gastrointestinal organs[2]. For example, several investigations have proved that there existed ectopic expression of PGC in breast, ovary, prostate and lung[3–6]. Unlike PGC, few studies are available concerning the ectopic expression of PGA. Up to now, only two reports have made mention of PGA expression outside the stomach. Meuwissen et al. proved the presence of PGA in Barrett's esophagus [7]. And another study conducted by Osman Mamat et al. demonstrated the expression of PGA in intraductal papillary mucinous neoplasms[8]. Further exploration of the relationship between PGA and extra-gastric diseases, especially cancer, could provide a more comprehensive understanding of the role and function of PGA.

Breast cancer in women has become the most common cancer worldwide and the fifth leading cause of cancer-related death[9]. It has been reported that some breast cancer cells and breast cysts epithelial cells could produce PGC[10]. Furthermore, several researches have suggested that PGC ectopic expression is closely related to the differentiation, biological behavior and prognosis of breast cancer[11–13]. However, it still remains unknown whether PGA is expressed in breast cancer tissue and its correlation with clinicopathological parameters of breast cancer.

In this study, we compared PGA expression in breast cancer and non-cancer tissues including different benign breast diseases by immunohistochemical staining, and further validation as well as function analysis was carried out with data from GEO to explore the role of PGA in breast cancer and its relationship with clinicopathological parameters as well as prognosis of breast cancer.

Materials And Methods

Collection of tissue specimens and clinical information

A total of 362 patients who underwent surgery between December 2016 and August 2017 at the department of breast surgery, the First Affiliated Hospital of China Medical University, were selected in this study. All the enrolled subjects were mainly separated into breast cancer group and non-cancer group including different benign diseases. The noncancer group were composed of patients with hyperplasia, fibroadenoma, mastopathy and other benign disease. Tissue samples were collected from all the participants. Basic information including age and menstruation status were taken by questionnaire. Clinical information concerning tumor size, tumor grade, lymph node metastasis, TNM stage, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor (HER2), proliferating cell nuclear antigen (Ki-67), and molecular classification were gathered from the electronic medical records of each participant. The study was approved by the Ethics Committee of the First Affiliated Hospital of China Medical University, and written informed consent was obtained from each patient.

Immunohistochemistry and its evaluation

The obtained tissue samples were fixed with formalin and embedded with paraffin. Each paraffin-embedded sample was cut into a 4-μm-thick section and mounted in a poly-L-lysine-coated glass slide for

preservation[14]. Immunohistochemistry (IHC) was performed as previously described[15]. PGA antibody (Cat: 13082-R021, rabbit, 1:1500 dilution; Sino Biological Inc., Beijing, China) was used in the study. Other reagents including citrate buffer (PH=6.0), phosphate- buffered saline (PBS, PH=7.4), secondary antibody and DAB (DAB-0031) stain were obtained from Maixin Inc., Fujian, China.

During the study, we controlled the quality of immunohistochemistry from three different aspects. Firstly, we used negative controls (PBS instead of primary and secondary antibodies) and positive controls during the experiment to avoid false negative or positive results. Secondly, the color reaction with DAB was observed under the microscope to prevent overstaining or under-staining. Thirdly, a double-blind method was applied to grade PGA expression levels. According to the double-blind principle, two pathologists observed and scored the expression of PGA protein in different sections independently. The staining intensity of each sample was graded on a scale of 0-3(I0-I3); the proportion of stained area was recorded as P(0-4): <5%(0), 6-25%(1), 26-50%(2), 51-75%(3), 76-100(4). For each tissue section, a HSCORE was calculated according to the following algorithm: $HSCORE = \sum(I \times P_i)$. And finally the stained results was classed into for groups according to the HSCORE: - (0); + (1-4); ++ (5-8); +++ (9-12).

Clinicopathological validation and prognostic evaluation of PGA with GEO database

RNA-seq data and related clinical information of PGA in breast cancer cohort were extracted from GEO database (GSE41119) (Gene Expression Omnibus; <https://www.ncbi.nlm.nih.gov/geo/>)[16]. The expression data were transformed by $\log_2$ [TPM (per million transcripts) +1]. Evaluation of the correlation between PGA expression and clinicopathological parameters with data from GEO was performed with R package ggpvr. Survival analysis with GEO data was conducted using R packages survival and survminer.

GO and KEGG enrichment analysis for PGA function and related pathway

Gene ontology (GO)[17] and Kyoto Encyclopedia of Genes and Genomes (KEGG)[18, 19] analysis are useful approaches to discover gene functions and related pathways. Firstly, we used R package limma to screen differentially expressed genes (DEGs) with data of GSE41119, which was separated into two groups according to the expression level of PGA by limma package too. And then GO and KEGG analysis was carried out with R package clusterProfiler and ggplot2 to illuminate the function and pathways of PGA.

Statistical analysis

Statistical analyses were performed by SPSS Statistics Version 20.0 (IBM, SPSS, and Chicago, IL, USA) and R software (R 4.0.4). The homogeneity of variance was tested for all data to determine the overall distribution type of data. Kruskal Wallis test was selected to estimate the differential expression of PGA among different groups. Receiver operating characteristic (ROC) curve was established to determine the diagnostic efficiency of PGA and to select the cut-off value that differentiated high and low expression.

The relationship between PGA protein expression and clinical characteristics of patients was evaluated by the chi-square test. Analysis with R packages was completed with the R software. P value no more than 0.05 was considered as significant.

Results

1. The PGA expression in breast cancer and non-cancer tissues

In this experiment, we compared the expression difference of PGA between breast cancer and non-cancer groups. The specific stained area was cytoplasm and representative photomicrographs of PGA expression in breast cancer and non-cancer groups were shown in Figure 1. The analysis results suggested that there was a significant statistical difference between the breast cancer and non-cancer group ($P<0.001$; Table 1).

Table 1
Expression of PGA in breast cancer and non-cancer tissues

	N	-	+	++	+++	P
Breast cancer	161	4	66	51	40	<0.001
Non-cancer	201	52	105	30	14	
Hyperplasia	24	12	10	2	0	P=0.255
Fibroadenoma	137	69	33	24	11	
Mastopathy	28	16	6	3	3	
Other benign disease	12	10	1	1	0	

2. Diagnostic efficiency of PGA expression for breast cancer

According to the analysis result of ROC curve, PGA protein expression (area=0.768; 95%CI = 0.720-0.816; $P<0.001$) was associated with incidence of breast cancer (Figure 2). And further we obtained an appropriate cut-off value which was 3.5 from the curve to define relative high and low expression of PGA. A total of 190 cases (52.4%) were high expression which above the average score. The positive rate in non-cancer group (71 cases, 35.3%) was lower than that in the breast cancer group (119 cases, 73.9%).

3. Correlations between PGA expression and clinical parameters of breast cancer

In this study, we explored the association between PGA protein expression and clinical features. Basic features of the study set were shown in supplementary table 1. The analysis results showed a statistical difference in the molecular classification of breast cancer (Table 2). To be specific, PGA expression in triple negative breast cancer (TNBC) was significantly different from the other three molecular types, of which were Luminal A ($P = 0.009$), Luminal B ($P = 0.034$), and HER2 ($P = 0.037$).

Table 2
PGA expression in cancer tissues

Tumor characteristics	Type	N	Low	High	P
Menstruation	Yes	73	21	67	0.756
	No	88	21	52	
Tumor size	≥2cm	131	31	100	0.114
	<2cm	29	11	18	
Tumor grade	I	21	4	17	0.455
	I & II	131	35	96	
Lymph Node Metastasis	YES	69	20	49	0.397
	NO	91	21	70	
TNM Stage	III & IV	26	9	17	0.291
	I & II	31	95	126	
ER	(-)	39	7	32	0.175
	(+)	121	35	86	
PR	(-)	58	11	47	0.136
	(+)	101	30	71	
HER-2	(-)	32	6	26	0.281
	(+)	128	36	92	
Ki-67	≥14%	129	32	97	0.397
	<14%	31	10	21	
Molecular classification	Luminal A	21	8	13	0.259 ¹
	Luminal B	67	17	50	
	HER2	11	3	8	0.540 ¹ ,0.894 ²
	TNBC	14	0	14	

0.009¹,0.034²,0.037³

Tumor characteristics	Type	N	Low	High	P
1 Compared with Luminal A					
2 Compared with Luminal B					
3 Compared with HER2					
TNBC: Triple negative breast cancer					
N: number					

4.Validation of clinical associations and evaluation of prognostic relevance

We used GEO data to verify the correlations between PGA and general clinical parameters. Our results suggested that there existed significant correlation of PGA expression between T0 and T1 ($P = 0.001$), T2 ($P = 0.004$), T3 ($P = 0.002$), T4 ($P = 0.020$), respectively (Figure 3D). As for tumor grade and molecular type, significant associations were found between grade 1 and grade 2 ($P = 0.018$; Fig. 3E), and between Luminal A type and unclassified type ($P = 0.017$; Fig. 3F). No significant correlation was found with age ($P = 0.790$; Fig. 3A), Er ($P = 0.290$; Fig. 3B), and Her2 ($P = 0.980$; Fig. 3C). In addition, we used GEO data to analyze the relationship between PGA expression and prognosis of breast cancer and the results showed that lower expression of PGA was associated with better prognosis of breast cancer ($P = 0.048$; Figure 4).

5. PGA function and related pathway

To further explore the possible functions and pathways of PGA in the breast, we further performed GO and KEGG enrichment analysis. First we identified DEGs associated with PGA expression levels with limma package. Heat maps of the first 20 different genes were shown in Figure 5. Then GO and KEGG analysis were performed with PGA and these DEGs. The GO analysis showed that PGA may be involved in nuclear division, regulation of lipid metabolic process and organelle fission (Figure 6A). Detailed analysis results of GO was exhibited in supplementary table. In KEGG enrichment analysis, PGA was closely related to PPAR signaling pathway and AMPK signaling pathway (Figure 6B).

Discussion

As important members of human pepsinogens families, PGA and PGC participated in the process of human digestion. Unlike PGC, relevant studies in PGA mainly focused on gastric diseases. There were few reports dealing with PGA expression outside the stomach. In the current research, we elucidated the expression status of PGA in breast cancer and non-cancer tissues with IHC and figured out its correlation with clinicopathological characteristics. Data from GEO was utilized for the verification of our results and for the confirmation of prognostic roles of PGA in breast cancer. And GO and KEGG analysis were performed to provide us a more comprehensive look at the functions of PGA.

First of all, we observed the expression of PGA in breast cancer and non-cancer tissues including different benign breast diseases by immunohistochemical staining and grouped all the cases by score results based on HSCORE. Diagnostic value of PGA in breast cancer was further investigated by ROC models. As revealed in our study, the expression of PGA was higher in breast cancer when compared with non-cancer tissues. In consistent with the expression trend of PGA in breast, Serra et al. observed in their experiment that PGC expression was negative in non-cancer breast tissues, whereas in tumor tissues the expression staining was statistically positive[20]. Analysis results of in situ expression of PGA in the stomach revealed that PGA was usually decreased in gastric cancer[21]. Besides, an earlier research implemented by Waalewijn RA et al. showed that in gastric cancer mRNA expressed level of PGA was obviously decreased[22]. Further ROC analysis demonstrated that PGA had potential to serve as a diagnostic marker of breast cancer, differentiating benign and malignant breast diseases. All the findings mentioned above indicated that there existed diverse expressional profiles of PGA in cancers originated from extra-gastric organs and stomach, and thus playing distinct roles in tumorigenesis of the two types of cancers.

Secondly, we analyzed the relationship between PGA expression and clinicopathological parameters and prognosis of breast cancer with IHC data and data from online database. IHC results showed that there was no significant statistical difference between PGA expression and general clinicopathological parameters of breast cancer except for the molecular classification. The overall positive rate of PGA expression was lower in Luminal A type and were at the middle level in Luminal B as well as HER-2 type, while the TNBC type showed more positive cases of PGA expression and more unique in comparison with other subtypes. Analysis with online data showed significant association of PGA with T stage, tumor grade and molecular type. Currently the molecular subtypes of breast cancer were identified mainly according to the expression of ER, PR, HER2, and Ki-67, which may be helpful for prognostic stratification and treatment selection[23]. TNBC is a special type of breast cancer with negative expression of ER, PR and HER2[24]. As an highly aggressive subtype of breast cancer, TNBC was reported to be associated with shorter survival time when compared with other subtypes[25–28]. Patients with TNBC had a more aggressive clinical course and a higher mortality rate[29, 30]. It was obvious that hormone or hormone receptor status, showed a highly closed correlation with prognosis of breast cancer patients. Survival analysis with data from GEO further proved that patients with increased levels of PGA had worse OS. Interestingly, PGC, which was also expressed as ectopic in the breast, had been reported that its expression in breast cancer was influenced by multiple hormones and receptors, thus showing different expression intensity and correlation with prognosis[31]. A former research conducted by Vizoso et al. proposed that downregulated expression of PGC was observed in poorly differentiated breast cancer patients and could be served as a novel prognostic marker for breast cancer[12]. Hence it could be hypothesized that possible interaction may exist between expression of PGA, ER, PR and HER2 simultaneously and subsequently exert an adverse influence on the prognosis of breast cancer patients. The inconsistent role of PGA and PGC in prognosis of breast cancer might due to the otherness lying in their regulation mechanisms in breast cancer. The findings of this study disclosed that higher PGA was observed in TNBC type and could be selected as a prognostic biomarker of breast cancer.

In order to comprehensively understand the potential function of PGA in breast cancer, we conducted bioinformatics analysis of GO and KEGG. The results revealed that PGA was primarily involved in nuclear division, regulation of lipid metabolic process and organelle fission. And KEGG further demonstrated that PGA was closely related to PPAR signaling pathway and AMPK signaling pathway. PPAR was a ligand-activated transcription factor belonging to the nuclear receptor superfamily[32]. Three subtypes of PPAR, α , β/δ and γ , were expressed in a tissue-specific manner in multiple species[33]. Multiple cells in the tumor microenvironment, including macrophages and fibroblasts, activation of PPAR γ induced a shift to a less aggressive phenotype that negatively affects breast cancer progression[34, 35]. In epithelial breast cancer cells, activation of PPAR γ resulted in decreased cell growth and motility, and increased autophagy and apoptosis[36]. Many studies had shown that autophagy can be activated by the AMPK signaling pathway, which was closely related to energy metabolism[37, 38]. AMPK was a key energy receptor that regulates cell energy homeostasis and could be activated by a variety of stimuli, particularly by factors which affected cell metabolism[39]. Reductions in AMPK activity were associated with altered cell metabolic processes that drove tumor growth and development, suggesting that AMPK acts as a metabolic tumor suppressor by reprogramming cell metabolism and inhibiting cell growth[40]. Activated AMPK phosphorylates several downstream target pathways that were involved in the regulation of a variety of cellular functions, such as cell proliferation, cell cycle progression, metabolism, and autophagy[41–45]. In our study, the expression of PGA was correlated with the molecular typing of breast cancer, and showed a certain trend in tumor growth and differentiation. In consideration of all these findings, we speculated that PGA may participate in the regulation of breast cancer through interactions with PPAR signaling pathway and AMPK signaling pathway.

Conclusion

In summary, in this study, for the first time, PGA was found to be expressed in breast tissue. The expression of PGA in breast cancer was significantly higher than that of non-cancer tissues including different benign diseases, and it was related to molecular classification of breast cancer. Survival analysis revealed that the prognosis of patients with higher PGA mRNA expression was poorer. PGA may function through interactions with PPAR signaling pathway and AMPK signaling pathway in breast cancer. This study lays a theoretical and experimental foundation for a more comprehensive understanding of the physiological and pathological functions of PGA from extra-gastric organ.

Declarations

Compliance with Ethical Standards

This study was approved by the Human Ethics Review Committee of the First Hospital of China Medical University (Shenyang, China), and written informed consent was obtained from each patients.

Consent for publication

All presentations of case reports have consent for publication.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interests.

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Authors'contributions

Y Yuan conceived the study. XD Lu completed experiments, data analysis and drafted the manuscript. QX contributed to sample collection and data analysis. J Chen contributed to bioinformatics analysis and drafting the manuscript. YZ Li partly participated in the literature search and bioinformatics data collection. YY reviewed the manuscript and made significance revisions on the drafts. All authors read and approved the final manuscript.

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References

1. Sano J, Miki K, Ichinose M, Kimura M, Kurokawa K, Aida T, Ishizaki M, Asano G, Masugi Y, Wong RN *et al*: **In situ localization of pepsinogens I and II mRNA in human gastric mucosa.** *Acta pathologica japonica* 1989, **39**(12):765-771.
2. Shen S, Jiang J, Yuan Y: **Pepsinogen C expression, regulation and its relationship with cancer.** *Cancer cell international* 2017, **17**:57.
3. Gonzalez LO, Corte MD, Junquera S, Bongera M, Rodriguez JC, Vizoso FJ: **Expression of androgen receptor and two androgen-induced proteins (apolipoprotein D and pepsinogen C) in ductal carcinoma in situ of the breast.** *Histopathology* 2007, **50**(7):866-874.
4. Rojo JV, Merino AM, Gonzalez LO, Vizoso F: **Expression and clinical significance of pepsinogen C in epithelial ovarian carcinomas.** *European journal of obstetrics, gynecology, and reproductive biology* 2002, **104**(1):58-63.
5. Diaz M, Rodriguez JC, Sanchez J, Sanchez MT, Martin A, Merino AM, Vizoso F: **Clinical significance of pepsinogen C tumor expression in patients with stage D2 prostate carcinoma.** *The International*

journal of biological markers 2002, **17**(2):125-129.

6. Foster C, Aktar A, Kopf D, Zhang P, Guttentag S: **Pepsinogen C: a type 2 cell-specific protease.** *American journal of physiology Lung cellular and molecular physiology* 2004, **286**(2):L382-387.
7. Meuwissen SG, Bosma A, van Donk E, Waalewijn R, Pals G, Pronk JC, Eriksson AW, Mullink H, Meijer CJ: **Immunohistochemical localization of pepsinogen A and C containing cells in Barrett's oesophagus.** *Virchows Archiv A, Pathological anatomy and histopathology* 1988, **413**(1):11-16.
8. Mamat O, Fukumura Y, Saito T, Takahashi M, Mitomi H, Sai JK, Kawasaki S, Yao T: **Fundic gland differentiation of oncocytic/pancreatobiliary subtypes of pancreatic intraductal papillary mucinous neoplasm.** *Histopathology* 2016, **69**(4):570-581.
9. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F: **Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.** *CA: a cancer journal for clinicians* 2021.
10. Serra Díaz C, Vizoso F, Rodríguez JC, Merino AM, González LO, Baltasar A, Pérez-Vázquez MT, Medrano J: **Expression of pepsinogen C in gynecomastias and male breast carcinomas.** *World journal of surgery* 1999, **23**(5):439-445.
11. Serra C, Vizoso F, Lamelas ML, Rodriguez JC, Gonzalez LO, Merino AM, Baltasar A, Perez-Vazquez MT, Medrano J: **Comparative study of two androgen-induced markers (apolipoprotein D and pepsinogen C) in female and male breast carcinoma.** *International journal of surgical investigation* 2000, **2**(3):183-192.
12. Vizoso F, Sanchez LM, Diez-Itza I, Merino AM, Lopez-Otin C: **Pepsinogen C is a new prognostic marker in primary breast cancer.** *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 1995, **13**(1):54-61.
13. Diez-Itza I, Merino AM, Tolivia J, Vizoso F, Sanchez LM, Lopez-Otin C: **Expression of pepsinogen C in human breast tumours and correlation with clinicopathologic parameters.** *British journal of cancer* 1993, **68**(3):637-640.
14. Deng N, Liu JW, Sun LP, Xu Q, Duan ZP, Dong NN, Yuan Y: **Expression of XPG protein in the development, progression and prognosis of gastric cancer.** *PloS one* 2014, **9**(9):e108704.
15. Jing JJ, Lu YZ, Sun LP, Liu JW, Gong YH, Xu Q, Dong NN, Yuan Y: **Epistatic SNP interaction of ERCC6 with ERCC8 and their joint protein expression contribute to gastric cancer/atrophic gastritis risk.** *Oncotarget* 2017, **8**(26):43140-43152.
16. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M *et al*: **NCBI GEO: archive for functional genomics data sets-update.** *Nucleic acids research* 2013, **41**(Database issue):D991-995.
17. **Expansion of the Gene Ontology knowledgebase and resources.** *Nucleic acids research* 2017, **45**(D1):D331-d338.
18. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K: **KEGG: new perspectives on genomes, pathways, diseases and drugs.** *Nucleic acids research* 2017, **45**(D1):D353-d361.

19. Kanehisa M, Goto S: **KEGG: kyoto encyclopedia of genes and genomes.** *Nucleic acids research* 2000, **28**(1):27-30.
20. **[Standardization for diagnostic tumor pathology: gastroenteropancreatic neuroendocrine neoplasm].** *Zhonghua Bing Li Xue Za Zhi* 2017, **46**(2):76-78.
21. Li P, He C, Sun L, Dong N, Yuan Y: **Pepsinogen I and II expressions in situ and their correlations with serum pepsinogen levels in gastric cancer and its precancerous disease.** *BMC Clin Pathol* 2013, **13**(1):22.
22. Waalewijn RA, Meuwissen SG, Pals G, Hoefsmit EC: **Localization of pepsinogen (A and C) and cellular differentiation of pepsinogen-synthesizing cells in the human gastric mucosa.** *Eur J Cell Biol* 1991, **54**(1):55-60.
23. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn HJ, Panel m: **Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011.** *Annals of oncology : official journal of the European Society for Medical Oncology* 2011, **22**(8):1736-1747.
24. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JM, Bilous M, Fitzgibbons P *et al*: **Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update.** *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2013, **31**(31):3997-4013.
25. Schneider BP, Winer EP, Foulkes WD, Garber J, Perou CM, Richardson A, Sledge GW, Carey LA: **Triple-negative breast cancer: risk factors to potential targets.** *Clinical cancer research : an official journal of the American Association for Cancer Research* 2008, **14**(24):8010-8018.
26. Foulkes WD, Stefansson IM, Chappuis PO, Begin LR, Goffin JR, Wong N, Trudel M, Akslen LA: **Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer.** *Journal of the National Cancer Institute* 2003, **95**(19):1482-1485.
27. Prat A, Cruz C, Hoadley KA, Diez O, Perou CM, Balmana J: **Molecular features of the basal-like breast cancer subtype based on BRCA1 mutation status.** *Breast cancer research and treatment* 2014, **147**(1):185-191.
28. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P, Narod SA: **Triple-negative breast cancer: clinical features and patterns of recurrence.** *Clinical cancer research : an official journal of the American Association for Cancer Research* 2007, **13**(15 Pt 1):4429-4434.
29. Zhang L, Fang C, Xu X, Li A, Cai Q, Long X: **Androgen receptor, EGFR, and BRCA1 as biomarkers in triple-negative breast cancer: a meta-analysis.** *BioMed research international* 2015, **2015**:357485.
30. Gluz O, Liedtke C, Gottschalk N, Pusztai L, Nitz U, Harbeck N: **Triple-negative breast cancer—current status and future directions.** *Annals of oncology : official journal of the European Society for Medical Oncology* 2009, **20**(12):1913-1927.

31. Scorilas A, Diamandis EP, Levesque MA, Papanastasiou-Diamandi A, Khosravi MJ, Giai M, Ponzone R, Roagna R, Sismondi P, Lopez-Otin C: **Immunoenzymatically determined pepsinogen C concentration in breast tumor cytosols: an independent favorable prognostic factor in node-positive patients.** *Clinical cancer research : an official journal of the American Association for Cancer Research* 1999, **5**(7):1778-1785.
32. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P *et al*: **The nuclear receptor superfamily: the second decade.** *Cell* 1995, **83**(6):835-839.
33. Bonofiglio D, Gabriele S, Aquila S, Catalano S, Gentile M, Middea E, Giordano F, Ando S: **Estrogen receptor alpha binds to peroxisome proliferator-activated receptor response element and negatively interferes with peroxisome proliferator-activated receptor gamma signaling in breast cancer cells.** *Clinical cancer research : an official journal of the American Association for Cancer Research* 2005, **11**(17):6139-6147.
34. Rovito D, Gionfriddo G, Barone I, Giordano C, Grande F, De Amicis F, Lanzino M, Catalano S, Ando S, Bonofiglio D: **Ligand-activated PPARgamma downregulates CXCR4 gene expression through a novel identified PPAR response element and inhibits breast cancer progression.** *Oncotarget* 2016, **7**(40):65109-65124.
35. Gionfriddo G, Plastina P, Augimeri G, Catalano S, Giordano C, Barone I, Morelli C, Giordano F, Gelsomino L, Sisci D *et al*: **Modulating Tumor-Associated Macrophage Polarization by Synthetic and Natural PPARgamma Ligands as a Potential Target in Breast Cancer.** *Cells* 2020, **9**(1).
36. Augimeri G, Gelsomino L, Plastina P, Giordano C, Barone I, Catalano S, Ando S, Bonofiglio D: **Natural and Synthetic PPARgamma Ligands in Tumor Microenvironment: A New Potential Strategy against Breast Cancer.** *International journal of molecular sciences* 2020, **21**(24).
37. Wu T, Wang MC, Jing L, Liu ZY, Guo H, Liu Y, Bai YY, Cheng YZ, Nan KJ, Liang X: **Autophagy facilitates lung adenocarcinoma resistance to cisplatin treatment by activation of AMPK/mTOR signaling pathway.** *Drug design, development and therapy* 2015, **9**:6421-6431.
38. Law BY, Mok SW, Chan WK, Xu SW, Wu AG, Yao XJ, Wang JR, Liu L, Wong VK: **Hernandezine, a novel AMPK activator induces autophagic cell death in drug-resistant cancers.** *Oncotarget* 2016, **7**(7):8090-8104.
39. Shang L, Chen S, Du F, Li S, Zhao L, Wang X: **Nutrient starvation elicits an acute autophagic response mediated by Ulk1 dephosphorylation and its subsequent dissociation from AMPK.** *Proceedings of the National Academy of Sciences of the United States of America* 2011, **108**(12):4788-4793.
40. Luo Z, Zang M, Guo W: **AMPK as a metabolic tumor suppressor: control of metabolism and cell growth.** *Future oncology (London, England)* 2010, **6**(3):457-470.
41. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, Shaw RJ: **AMPK phosphorylation of raptor mediates a metabolic checkpoint.** *Molecular cell* 2008, **30**(2):214-226.
42. Kim J, Kundu M, Viollet B, Guan KL: **AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1.** *Nature cell biology* 2011, **13**(2):132-141.

43. Mack HI, Zheng B, Asara JM, Thomas SM: **AMPK-dependent phosphorylation of ULK1 regulates ATG9 localization.** *Autophagy* 2012, **8**(8):1197-1214.
44. Hardie DG: **AMPK: a target for drugs and natural products with effects on both diabetes and cancer.** *Diabetes* 2013, **62**(7):2164-2172.
45. He G, Zhang YW, Lee JH, Zeng SX, Wang YV, Luo Z, Dong XC, Viollet B, Wahl GM, Lu H: **AMP-activated protein kinase induces p53 by phosphorylating MDMX and inhibiting its activity.** *Molecular and cellular biology* 2014, **34**(2):148-157.

Supplementary

Supplementary Table 1 is not available with this version

Figures

Figure 1

Representative photomicrographs of PGA expression in breast cancer and non-cancer groups.

A. breast cancer with negative PGA expression; B. breast cancer with positive PGA expression; C. Non-cancer with negative PGA expression; D. Non-cancer with positive PGA expression. (bar = 40μm; magnification ×200, by MOTIC digital slice scanning and application system)

Figure 2

Diagnostic value of PGA in breast cancer.

ROC of PGA in breast cancer showed that elevated expression of PGA was correlated with incidence of breast cancer.

Figure 3

Correlation between PGA expression and general clinical parameters in database

A. age; B. Er; C. Her2; D. T stage; E. tumor grade; F. molecular type.

Figure 4

Effect of PGA expression on prognosis of patients with breast cancer

Figure 5

Heat map of DEGs associated with PGA expressed levels

Figure 6

Enrichment analysis results of GO and KEGG

A. GO enrichment analysis; B. KEGG enrichment analysis.