Tumor Co-Expression of Progranulin and Sortilin Identifies a Highly Malignant Subgroup of Breast Cancer

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Abstract

Background

The growth factor progranulin has been implicated in numerous biological processes such as wound healing, inflammation and progressive tumorigenesis. Both progranulin and its receptor sortilin are known to be highly expressed in subgroups of breast cancer and have been associated with various clinical properties including tamoxifen resistance. Recent data further suggest that progranulin via its receptor sortilin can drive breast cancer stem cell propagation as well as increased metastasis formation in an *in vivo* breast cancer xenograft model. In this retrospective biomarker analysis, we aimed to determine whether tumor co-expression of progranulin and sortilin has prognostic and treatment predictive values for breast cancer patients.

Patients and Methods

We explored how co-expression of progranulin and sortilin was associated with established clinical markers by analyzing a tissue microarray including 560 randomized premenopausal breast cancer patients receiving either two years of tamoxifen treatment or no adjuvant treatment, with a median follow-up time of 28 years. Breast cancer-specific survival were analyzed with Kaplan-Meier and Cox Proportional Hazards regression models to assess the prognostic and predictive value of progranulin and sortilin in relation to known clinical markers.

Results

Co-expression of progranulin and sortilin was observed in 20% of the breast cancer samples. In untreated patients, prognostic considerations could be detailed separately from treatment prediction and the high progranulin and sortilin expressing subgroup was significantly associated with breast cancer-specific death in multivariable analyses (HR=2.188, CI: 1.317-3.637, $p=0.003$) along with tumor size, high tumor grade and lymph node positivity. When comparing the untreated patients with tamoxifen treated patients in the ER α positive subgroup, co-expression of progranulin and sortilin was not linked to tamoxifen resistance.

Conclusion

Data suggests that co-expression of progranulin and its receptor sortilin is a novel prognostic biomarker combination identifying a highly malignant subgroup of breast cancer. Importantly, this subpopulation could potentially be targeted with anti-sortilin based therapies.

Background

Breast cancer is the most common cancer in women worldwide. Even though early detection rates and existing therapies contributes to a slightly enhanced survival, many patients experience metastasis and tumor relapse. As a result, breast cancer remains the leading cause of cancer death among the female

population(1). Treatment failure and cancer relapses are multifactorial but most likely influenced by drug resistance and self-renewal properties of the small population of tumor cells termed cancer stem cells (CSCs)(2). Further, breast cancer is in many aspects a heterogeneous disease, including subtypes with diverse phenotypes and clinical behaviors(3-5). Each of the subtypes is responsive to different treatment regimes. Patients that express the estrogen receptor alpha (ERα) are treated with endocrine adjuvant therapy, such as tamoxifen or an aromatase inhibitor. However, despite that endocrine therapy improves the survival of the ERα positive patient group, many patients experience tumor relapse or therapy resistance(6). Consequently, there is an obvious need to further identify key mediators involved in breast cancer progression in order to optimally distinguish subgroups of breast cancer patients that will benefit from specific treatments or having tumors with inherent aggressive properties.

The pleiotropic growth factor progranulin has been identified as a key mediator involved in breast cancer progression and is further influenced by the tumor microenvironment, which can lead to breast CSC propagation and drug resistance(7-12). We recently observed that progranulin secretion is induced from ERα positive breast cancer cells in hypoxic environment, which further induced breast CSC propagation(7). Progranulin is an autocrine growth factor involved in various biological processes, such as wound healing, tumorigenesis, inflammation, as well as various neurological diseases(13-20). The fact that high progranulin has been observed in both tissue and serum of various cancer types compared to normal tissue(9, 12, 21-28) suggests that progranulin may be a relevant biomarker in breast cancer, as well as in other cancer types(28, 29).

Moreover, sortilin is a known progranulin binding receptor(30), highly expressed in breast cancer cell lines compared to non-tumorigenic breast epithelial cells(31). Sortilin has also been associated with increased metastatic potential in breast cancer(31), suggesting that the progranulin receptor could also be involved in breast cancer progression.

Here, we investigated tumor specific expression and potential clinical associations for progranulin and its associated receptor sortilin, using tissue microarrays (TMAs) from a randomized tamoxifen trial including 560 premenopausal breast cancer patients with the aim to identify optimal biomarkers for breast cancer progression and prognosis that could potentially be targeted by anti-sortilin based therapy. This study was performed according to REMARK guidelines presented in Additional file 1.

Materials And Methods

Ethics Statement

The original study (SBII:2) was approved by the Ethics Committee at Lund and Linköping Universities, Sweden (Dnr LU 240-01 and for the continuation of the study: Dnr Linköping 01-134 and Dnr LU 2015-350). Randomization was performed by the Regional Oncological Centers and oral informed consent was registered for all patients. The data was analyzed anonymously.

Patients and tumor samples

This retrospective study includes an invasive breast cancer cohort consisting of 560 premenopausal patients enrolled in a randomized clinical trial from 1984-1991, where patients received either two years of tamoxifen treatment ($n=275$) or no systemic treatment (randomized untreated) ($n=285$). Each patient underwent surgery (either radical mastectomy or breast-conserving surgery) followed by radiotherapy and in a small number of cases adjuvant polychemotherapy (>2%). All patients were followed-up for breast cancer-specific survival (BCSS) with up to 32 years of follow-up data. BCSS was calculated as the time from surgery of primary breast cancer to death from breast cancer. The median post-surgery follow-up time without a breast cancer-specific death was 28.41 years. ER α status was determined by immunohistochemistry or enzyme immunoassay, progesterone receptor (PR) status by immunohistochemistry and human epidermal growth factor receptor 2 (HER2) status was determined by *in situ* hybridization and immunohistochemistry as previously described(32, 33). A CONSORT diagram for the trial profile is given in Additional file 2: Figure S1 and clinical and tumor characteristics for the two study groups are presented in Table 1. Additional details of the trial have been described previously(32, 34, 35).

Immunohistochemistry

Representative tumor areas of formalin-fixed and paraffin-embedded tissue material was collected from 444 of the 560 patients and selected for TMA construction and sectioned, followed by deparaffinization and rehydration as previously described(34). Progranulin and sortilin expression were determined by immunohistochemistry using an Autostainer LINK 48 and the Envision FLEX+ detection system (DAKO). Deparaffinized sections (4.5 μ m) were subjected to antigen retrieval by high pressure cooking and DIVA antigen retrieval pH 6.2, followed by blocking with 3% hydrogen peroxide and incubation with primary antibody against progranulin (polyclonal goat anti-Progranulin, #AF2420, R&D Systems 1:1000) and sortilin (polyclonal rabbit anti-Sortilin, #AB16640, ABCAM 1:1000) at room temperature for 1 hour. A secondary antibody (polyclonal rabbit anti-goat immunoglobulins/HRP, #P0449, DAKO 1:100) was used for the progranulin staining. For signal amplification, EnVision™ FLEX+ Rabbit linker (SM805, DAKO) was used. Further, the EnVision FLEX/HRP visualization reagent EnVision™ FLEX/HRP secondary antibody-coated polymer peroxidase complexes (#SM802, DAKO) was used, followed by DAB substrate/chromogen (DAKO). Slides were counterstained with hematoxylin (DAKO) and stained sections were scanned by Leica SCN400 scanner at 20X. Antibody validation for IHC assessment of sortilin and progranulin was performed using established breast cancer cell models (Additional file 4 and 5).

Scoring

Scoring was performed independently by pathologists (Landberg) and a trained breast cancer surgeon (Rafnsdóttir) without knowledge of pathological or clinical data. The immunostaining scoring for progranulin and sortilin were implemented using an Allred scoring system, ranging from 1 (no/low staining), 2 (low/intermediate staining), 3 (intermediate/high staining) to 4 (high staining).

Statistical analysis

All statistical calculations and modelling were performed in SPSS software version 25 (SPSS, Chicago, IL), GraphPad Prism version 7.00 (GraphPad Software, San Diego, CA) or RStudio version 3.6.2 (packages *stats*, *ggplot2*, *rms*, *survival* and *survminer*). Spearman's rank-order Correlation Coefficient was used to test the significance of the association between progranulin and sortilin scoring. The relationship between progranulin and sortilin scoring and various parameters were analyzed using Pearson's Chi-square test for categorical variables and Kruskal-Wallis (or Man-Whitney U) test for continuous variables. Kaplan-Meier curves were used to estimate BCSS, and the log-rank test was used to compare BCSS among different staining and treatments, as well as to calculate Hazard Ratio (HR) and 95% Confidence Interval (CI) in these groups. Univariate and multivariable analysis was performed using Cox proportional hazard model for relative risk estimation of different variables, including tumor grade, size, age, lymph node status and ER α status to compare BCSS among different treatment groups. For univariate and multivariable analysis, HR and 95% CI were calculated. Performance of the multivariable models were measured using the concordance index (C-index) and the proportional hazards assumption was tested by Schoenfeld residuals. A 10-fold cross-validation, repeated 100 times, were performed to validate the cohort and estimate the prediction accuracy of the fitted model. All *p*-values correspond to two-sided tests, and *p*-values of < 0.05 were considered statistically significant.

Results

Correlation between progranulin and sortilin expression and clinicopathological parameters

In order to validate the potential prognostic as well as treatment predictive value of progranulin and sortilin tumor expression, we analyzed 444 breast cancer samples arranged in TMAs that were successfully stained for progranulin and sortilin using immunohistochemistry (IHC). Clinicopathological and molecular parameters included in the study are summarized in Table 1. The median age of the patients was 45 years (range 25-57) and the median follow-up period was 28.41 years. At the last follow-up, 206 (46.4%) of the 444 patients analyzed had died of breast cancer. To predict the accuracy of the multivariable model, a 10-fold cross-validation, repeated 100 times, were performed and demonstrated equivalent results (C-index: 0.642 for all patients in the full model and mean C-index of the repeated test-sets: 0.616). For the cohort studied, there was a significantly increased BCSS for tamoxifen treated patients having ER α positive cancer (*p*=0.031, *n*=384) (see Additional file 2: Figure S2).

Breast cancer-specific progranulin and sortilin protein expression were scored into four groups as illustrated in Figure 1 and further subdivided into low expression (score 1-2) or high expression (score 3-4). Among the 444 primary breast tumors selected, 412 tumors were successfully stained for progranulin where 273 tumors (66.26%) were categorized as having low progranulin levels (score 1-2) and 139 tumors (33.74%) had high progranulin expression (score 3-4). For sortilin expression analysis, 427 of the 444 breast tumors were successfully stained and 225 tumors had high sortilin expression (score 3-4) (52.69%) and 202 tumors had low expression of sortilin (47.31%) (score 1-2) (Table 1).

Next, we investigated how progranulin and sortilin protein expression were associated with established clinicopathological parameters. In support for a biological association, progranulin and sortilin protein expression correlated significantly ($r=0.112$, $p=0.026$) (see Additional file 2: Table S1). Further, progranulin tumor expression was significantly linked to histological grade ($p<0.001$), where patients with high-grade tumors showed high progranulin expression as well as Ki67 ($p=0.001$) and the hypoxic marker hypoxia-inducible factor 1-alpha (HIF1 α) ($p=0.002$) (see Additional file 2: Table S1). There was also a significant association between progranulin and ER α status ($p<0.001$) as well as PR status ($p=0.001$) (see Additional file 2: Table S1), where ER α positive tumors tend to have lower progranulin expression, which is consistent with previous findings(16). In addition, sortilin expression was significantly associated with ER α , where ER α positive tumors tend to have higher sortilin ($p=0.004$) and PR expression ($p<0.001$). Further, age was also significantly linked to sortilin expression ($p=0.040$) (see Additional file 2: Table S2).

Patients with high tumor co-expression of progranulin and sortilin had impaired BCSS

Since the aim of this study was to evaluate the aggressiveness of breast cancers expressing both progranulin and the receptor sortilin, the material was subdivided into four groups based on progranulin and sortilin co-expression: 1; low progranulin/low sortilin, 2; low progranulin/high sortilin, 3; high progranulin/low sortilin and 4; high progranulin/high sortilin (see Additional file 2: Table S3). Out of 395 scored tumors, 79 (20 %) expressed high levels of both progranulin and sortilin, 56 (14.18%) had high progranulin/low sortilin expression, 129 (32.66%) had high sortilin/low progranulin, and 131 (33.16%) expressed low levels of both markers.

For the analysis of progranulin and sortilin expression in relation to BCSS, we initially concentrated on the randomized untreated patients in order to obtain prognostic information not affected by adjuvant tamoxifen treatment (all univariate data Table 2, left). Interestingly, the double high progranulin and sortilin group was significantly different from the remaining subgroups and also associated with worse outcome, as illustrated in Figure 2A ($p=0.003$, $n=206$). When indicating all four subgroups, the double high subgroup separated significantly from the two subgroups of low progranulin expression ($p=0.021$ and $p=0.005$) whereas there was a non-significant trend for a difference between the double high group and progranulin high group with low sortilin expression ($p=0.170$) (Figure 2B). In order to clarify the significance of adding sortilin expression to progranulin, we performed multivariable Cox Proportional Hazard (CPH) regression analyses, only analyzing patients with high progranulin tumor expression. In support for an important additive function for sortilin in the progranulin high patient group, high tumor tissue expression of sortilin was significantly linked to BCSS (HR=3.013, 95% CI: 1.219-7.448, $p=0.017$) together with lymph node (LN) positivity (HR=3.854, 95% CI: 1.666-8.919, $p=0.002$) and tumor size (HR=1.089, 95% CI: 1.037-1.143, $p=0.001$) (C-index: 0.701) (see Additional file 2: Table S4).

The univariate analysis (Table 2), linking the double high progranulin and sortilin to reduced BCSS in untreated patients was further validated by multivariable CPH regression analysis in the two subsets of patients including progranulin and sortilin co-expression as well as regular prognostic parameters available for the study. Results showed that high progranulin and sortilin co-expression together with

grade, tumor size and LN status were identified as significant risk factors for BCSS (double high: HR=2.188, 95% CI: 1.317-3.637, $p=0.003$, high grade: HR=1.737, 95% CI: 1.054-2.860, $p=0.030$, LN positivity; HR=2.250, 95% CI: 1.348-3.758, $p=0.002$, respectively) (C-index: 0.667) (Table 2, right). Next, we included all patients available within the randomized study in order to increase the statistical power of the data. In this extended patient material, including tamoxifen treated patients, high co-expression of progranulin and sortilin was significantly linked to BCSS ($p=0.003$, $n=395$) as illustrated in Additional file 2: Figure S3. In addition, univariate and multivariable CPH analysis on all patients revealed comparable results as for the untreated patients (C-index: 0.642) (see Additional file 2: Table S5). Here, endocrine treatment with tamoxifen was also identified as an independent prognostic variable (HR=0.710, 95% CI: 0.517-0.974, $p=0.034$). Interestingly, the double high group was not associated with any of the established clinicopathological parameters, including grade ($p=0.063$) and Ki67 ($p=0.066$) (see Additional file 2: Table S6).

High tumor co-expression of progranulin and sortilin was not associated with tamoxifen resistance

Since the analyzed cohort include randomized untreated and tamoxifen treated patients we could define a potential tamoxifen response or resistance in the subgroup of patients with high co-expression of progranulin and sortilin. These analyses were restricted to patients with ER α positive breast cancer where high tumor co-expression of progranulin and sortilin demonstrated a significantly worse BCSS compared to mixed groups ($p=0.005$, $n=279$) (Figure 3A) similar to all samples described above. Multivariable analysis revealed that the double high group (HR=1.980, 95% CI: 1.308-2.996, $p=0.001$) as well as grade (HR=1.612, 95% CI: 1.086-2.394, $p=0.018$) and HER2 positivity (HR=1.716, 95% CI: 1.027-2.867, $p=0.039$) were significantly associated with reduced BCSS in ER α positive breast cancer and that tamoxifen treatment significantly improved ER α positive BCSS (HR=0.628, 95% CI: 0.431-0.915, $p=0.015$) (C-index: 0.637) (see Additional file 2: Table S7). When analyzing the response to tamoxifen treatment, the ER α positive patients with double high expression revealed no significant improvement in BCSS comparing untreated patients with the tamoxifen treated group ($p=0.231$, $n=55$) (Figure 3B) in contrast to the remaining group with mixed progranulin and sortilin expression ($p=0.046$, $n=224$) (Figure 3C). This potential difference in tamoxifen response was nevertheless not significant in an interaction analysis ($p=0.971$), suggesting that despite the lack of significant response to tamoxifen treatment in univariate analysis, the double high group was not resistant to tamoxifen treatment. Altogether, these results suggest that high co-expression of progranulin and sortilin recognizes an ER α positive patient group that could benefit from complementary therapy, targeting sortilin.

Discussion

Despite valuable traditional cancer therapies, many breast cancer patients experience relapse and therapy resistance. Thus, it is vital to continue to search for mediators driving tumor progression as well as identify biomarkers that better predict high-risk patients with a more aggressive clinical behavior as well as potential resistance to various therapies. The interest in progranulin has emerged over the last years, with publications demonstrating an overexpression of progranulin in different cancer types and

associations with poor prognosis and survival(9, 12, 21-28). Further, the progranulin receptor sortilin has been linked to breast cancer aggressiveness as well as being expressed in other types of cancer, such as prostate and ovarian cancer(31, 36, 37). Recent studies from our group have emphasized the stem cell propagating effect by progranulin through its receptor sortilin(7), indicating that this pathway could be central in mediating CSC properties during tumor progression. The existence of a targetable receptor further suggest that future cancer therapies could be developed, selectively targeting CSC propagation via sortilin.

Here, we analyzed the expression levels of both progranulin and its receptor sortilin in a large and unique randomized clinical trial with long-term follow-up in order to clarify if tumor co-expression of the activator and the receptor define any specific breast cancer type in relation to clinical aggressiveness. The results indeed showed that high co-expression of progranulin and sortilin could be detected in 20% of the patients and was associated with decreased BCSS. In support for an important function of progranulin and sortilin activation in breast cancer progression, multivariable regression analysis identified high co-expression of progranulin and sortilin, as well as histological grade and lymph node status as independent risk factors.

Previous reports have associated high progranulin expression with ER α negative patients(16) as well as a predictive marker for recurrence in ER α positive breast cancer(29). We recently showed that progranulin secretion increased in ER α positive breast cancer when cells were subjected to hypoxia, whereas ER α negative breast cancer cells had constitutive high secretion of progranulin(7). Here we observed a significant link between progranulin and HIF1 α , where tumors with high expression of HIF1 α tend to express high progranulin. The positive link of progranulin expression with HIF1 α suggests a hypoxic influence on progranulin expression, which is in line with previous published data(7). Further, we observed that high progranulin expression tend to associate with ER α negative status. In contrast, high sortilin expression was associated with ER α positive tumors, which suggests that even though progranulin associated with sortilin, their respective link to ER α are different. Although, the clinical relevance of hypoxic driven progranulin induced CSC propagation in different breast cancer subtypes needs to be studied further.

Sortilin has previously been associated with breast cancer aggressiveness and contributes to tumor cell adhesion and invasion(38). We recently published that a small molecule inhibitor of sortilin (AF38469) (39) block progranulin induced breast cancer progression *in vivo*(7) . In this study, orally administration of AF38469 significantly reduced the development of metastasis, which suggest that sortilin may function as a therapeutic target in breast cancer.

Here, in this cohort, high sortilin tumor expression on its own demonstrated no significant reduction of BCSS in either all patients or in the randomized control group (a Kaplan-Meier plot of the sortilin expression is shown in Additional file 3). However, the combination of high tumor co-expression of progranulin and sortilin demonstrated a significantly worse BCSS. Importantly, multivariable analysis revealed that when analyzing the progranulin high subgroup separately, high sortilin expression was

identified as a significant prognostic variate linked to worse BCSS together with lymph node positivity and tumor size. This support the fact that sortilin add prognostic information when combined with progranulin.

Current treatment for patients with ER α positive tumors includes endocrine therapy such as tamoxifen and a previous report described progranulin to be associated with resistance towards tamoxifen therapy(8). Further, another report suggested that progranulin levels predicted recurrence in patients with hormone receptor positive breast cancer during tamoxifen treatment(28). Here, multivariable interaction analysis identified that the double progranulin/sortilin high ER α positive group was not resistance to tamoxifen treatment, even though univariate analysis demonstrated no significant improvement in BCSS in the tamoxifen treated group. Notably, the tamoxifen treatment in this cohort is only two years of adjuvant treatment and BCSS may also have been affected by later therapies at disease recurrence.

Conclusion

In conclusion, we have shown that a combination of high progranulin and high sortilin tumor tissue expression defines a novel and highly malignant subgroup of breast cancer patients that can be targeted by anti-sortilin based therapies.

Abbreviations

BCSS: breast cancer-specific survival

C-index: concordance index

CI: confidence interval

CPH: cox proportional hazard

CSC: cancer stem cell

ER α : estrogen receptor alpha

HER2: human epidermal growth factor receptor 2

HIF1 α : hypoxia-inducible factor 1-alpha

IHC: immunohistochemistry

LN: lymph node

PR: progesterone receptor

TMA: tissue microarray

Declarations

Author Contributions

Concept and design: KB, SRh, ÉH, GL. Execution of experiments: YM, SRa. Analysis and interpretation of the data: KB, SRh, GL. Contribution of reagents/materials: LR, OS, ME. Drafting of the manuscript: KB, SRh, GL. Critical revision of the manuscript: KB, SRh, GL, LR, OS, ÉH, SRa, YM, ME.

Conflict of Interest Disclosures

None reported.

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Role of the Funder/Sponsor

The funders had no role in the design and conduct of the study; data collection and analysis; decision to publish, or preparation of the manuscript.

Additional Contributions

We thank Björn Andersson and Jari Martikainen from the Bioinformatics Core Facilities at the Sahlgrenska Academy for statistical/bioinformatics support.

Availability of data and materials

The dataset analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The original study (SBII:2) was approved by the Ethics Committee at Lund and Linköping Universities, Sweden (Dnr LU 240-01 and for the continuation of the study: Dnr Linköping 01-134 and Dnr LU 2015-350). Randomization was performed by the Regional Oncological Centers and oral informed consent was registered for all patients.

Consent for publication

All authors read and approved the final manuscript.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424.
2. Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature*. 2009;458:780.
3. Meacham CE, Morrison SJ. Tumour heterogeneity and cancer cell plasticity. *Nature*. 2013;501:328.
4. Axelson H, Fredlund E, Ovenberger M, Landberg G, Pahlman S. Hypoxia-induced dedifferentiation of tumor cells—a mechanism behind heterogeneity and aggressiveness of solid tumors. *Semin Cell Dev Biol*. 2005;16(4-5):554-63.
5. Conley SJ, Gheordunescu E, Kakarala P, Newman B, Korkaya H, Heath AN, et al. Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. *Proc Natl Acad Sci U S A*. 2012;109(8):2784-9.
6. Zelnak AB, O'Regan RM. Optimizing Endocrine Therapy for Breast Cancer. *J Natl Compr Canc Netw*. 2015;13(8):e56-64.
7. Rhost S, Hughes E, Harrison H, Rafnsdottir S, Jacobsson H, Gregersson P, et al. Sortilin inhibition limits secretion-induced progranulin-dependent breast cancer progression and cancer stem cell expansion. *Breast Cancer Res*. 2018;20(1):137.
8. Tangkeangsirisin W, Hayashi J, Serrero G. PC Cell-Derived Growth Factor Mediates Tamoxifen Resistance and Promotes Tumor Growth of Human Breast Cancer Cells. *Cancer Res*. 2004;64(5):1737-43.
9. Wang M, Li G, Yin J, Lin T, Zhang J. Progranulin overexpression predicts overall survival in patients with glioblastoma. *Med Oncol*. 2012;29(4):2423-31.
10. Kim WE, Serrero G. PC cell-derived growth factor stimulates proliferation and confers Trastuzumab resistance to Her-2-overexpressing breast cancer cells. *Clin Cancer Res*. 2006;12(14 Pt 1):4192-9.
11. Abrhale T, Brodie A, Sabnis G, Macedo L, Tian C, Yue B, et al. GP88 (PC-Cell Derived Growth Factor, progranulin) stimulates proliferation and confers letrozole resistance to aromatase overexpressing breast cancer cells. *BMC Cancer*. 2011;11:231-.
12. Pizarro GO, Zhou XC, Koch A, Gharib M, Raval S, Bible K, et al. Prosurvival function of the granulin-epithelin precursor is important in tumor progression and chemoresponse. *Int J Cancer*. 2007;120(11):2339-43.
13. De Muynck L, Van Damme P. Cellular Effects of Progranulin in Health and Disease. *J Mol Neurosci*. 2011;45(3):549.
14. Abella V, Pino J, Scotece M, Conde J, Lago F, Gonzalez-Gay MA, et al. Progranulin as a biomarker and potential therapeutic agent. *Drug Discovery Today*. 2017;22(10):1557-64.
15. Arechavaleta-Velasco F, Perez-Juarez CE, Gerton GL, Diaz-Cueto L. Progranulin and its biological effects in cancer. *Med Oncol*. 2017;34(12):194.

16. Lu R, Serrero G. Inhibition of PC cell-derived growth factor (PCDGF, epithelin/granulin precursor) expression by antisense PCDGF cDNA transfection inhibits tumorigenicity of the human breast carcinoma cell line MDA-MB-468. *Proc Natl Acad Sci U S A*. 2000;97(8):3993-8.
17. Ong CH, Bateman A. Progranulin (granulin-epithelin precursor, PC-cell derived growth factor, acrogranin) in proliferation and tumorigenesis. *Histol Histopathol*. 2003;18(4):1275-88.
18. He Z, Ong CH, Halper J, Bateman A. Progranulin is a mediator of the wound response. *Nat Med*. 2003;9(2):225-9.
19. Cenik B, Sephton CF, Kutluk Cenik B, Herz J, Yu G. Progranulin: A Proteolytically Processed Protein at the Crossroads of Inflammation and Neurodegeneration. *The Journal of Biological Chemistry*. 2012;287(39):32298-306.
20. Petkau TL, Leavitt BR. Progranulin in neurodegenerative disease. *Trends Neurosci*. 2014;37(7):388-98.
21. Serrero G, Ioffe OB. Expression of PC-cell-derived growth factor in benign and malignant human breast epithelium. *Hum Pathol*. 2003;34(11):1148-54.
22. Edelman MJ, Feliciano J, Yue B, Bejarano P, Ioffe O, Reisman D, et al. GP88 (Progranulin): A novel tissue and circulating biomarker for non-small cell lung carcinoma. *Hum Pathol*. 2014;45(9):1893-9.
23. Yamamoto Y, Goto N, Takemura M, Yamasuge W, Yabe K, Takami T, et al. Association between increased serum GP88 (progranulin) concentrations and prognosis in patients with malignant lymphomas. *Clin Chim Acta*. 2017;473:139-46.
24. Göbel M, Eisele L, Möllmann M, Hüttmann A, Johansson P, Scholtysik R, et al. Progranulin Is a Novel Independent Predictor of Disease Progression and Overall Survival in Chronic Lymphocytic Leukemia. *PLoS One*. 2013;8(8):e72107.
25. Pan C-X, Kinch MS, Kiener PA, Langermann S, Serrero G, Sun L, et al. PC Cell-Derived Growth Factor Expression in Prostatic Intraepithelial Neoplasia and Prostatic Adenocarcinoma. *Clin Cancer Res*. 2004;10(4):1333.
26. Tkaczuk KR, Yue B, Zhan M, Tait N, Yarlagadda L, Dai H, et al. Increased Circulating Level of the Survival Factor GP88 (Progranulin) in the Serum of Breast Cancer Patients When Compared to Healthy Subjects. *Breast Cancer : Basic and Clinical Research*. 2011;5:155-62.
27. Han JJ, Yu M, Houston N, Steinberg SM, Kohn EC. Progranulin is a potential prognostic biomarker in advanced epithelial ovarian cancers. *Gynecol Oncol*. 2011;120(1):5-10.
28. Koo DH, Park C-Y, Lee ES, Ro J, Oh SW. Progranulin as a Prognostic Biomarker for Breast Cancer Recurrence in Patients Who Had Hormone Receptor-Positive Tumors: A Cohort Study. *PLoS One*. 2012;7(6):e39880.
29. Serrero G, Hawkins DM, Yue B, Ioffe O, Bejarano P, Phillips JT, et al. Progranulin (GP88) tumor tissue expression is associated with increased risk of recurrence in breast cancer patients diagnosed with estrogen receptor positive invasive ductal carcinoma. *Breast Cancer Research : BCR*. 2012;14(1):R26-R.

30. Lee WC, Almeida S, Prudencio M, Caulfield TR, Zhang Y-J, Tay WM, et al. Targeted manipulation of the sortilin–progranulin axis rescues progranulin haploinsufficiency. *Human Molecular Genetics*. 2013.
31. Roselli S, Pundavela J, Demont Y, Faulkner S, Keene S, Attia J, et al. Sortilin is associated with breast cancer aggressiveness and contributes to tumor cell adhesion and invasion. *Oncotarget*. 2015;6(12):10473-86.
32. Rydén L, Jönsson P-E, Chebil G, Dufmats M, Fernö M, Jirström K, et al. Two years of adjuvant tamoxifen in premenopausal patients with breast cancer: a randomised, controlled trial with long-term follow-up. *Eur J Cancer*. 2005;41(2):256-64.
33. Rydén L, Landberg G, Stål O, Nordenskjöld B, Fernö M, Bendahl P-O. HER2 status in hormone receptor positive premenopausal primary breast cancer adds prognostic, but not tamoxifen treatment predictive, information. *Breast Cancer Res Treat*. 2008;109(2):351-7.
34. Holm C, Rayala S, Jirström K, Stål O, Kumar R, Landberg G. Association Between Pak1 Expression and Subcellular Localization and Tamoxifen Resistance in Breast Cancer Patients. *JNCI: Journal of the National Cancer Institute*. 2006;98(10):671-80.
35. Ekholm M, Bendahl P-O, Fernö M, Nordenskjöld B, Stål O, Rydén L. Two Years of Adjuvant Tamoxifen Provides a Survival Benefit Compared With No Systemic Treatment in Premenopausal Patients With Primary Breast Cancer: Long-Term Follow-Up (> 25 years) of the Phase III SBII:2pre Trial. *J Clin Oncol*. 2016;34(19):2232-8.
36. Ghaemimanesh F, Ahmadian G, Talebi S, Zarnani A-H, Behmanesh M, Hemmati S, et al. The effect of sortilin silencing on ovarian carcinoma cells. *Avicenna journal of medical biotechnology*. 2014;6(3):169-77.
37. Tanimoto R, Morcavallo A, Terracciano M, Xu S-Q, Stefanello M, Buraschi S, et al. Sortilin regulates progranulin action in castration-resistant prostate cancer cells. *Endocrinology*. 2015;156(1):58-70.
38. Roselli S, Pundavela J, Demont Y, Faulkner S, Keene S, Attia J, et al. Sortilin is associated with breast cancer aggressiveness and contributes to tumor cell adhesion and invasion. *Oncotarget*. 2015;6(12):10473-86.
39. Schroder TJ, Christensen S, Lindberg S, Langgard M, David L, Maltas PJ, et al. The identification of AF38469: an orally bioavailable inhibitor of the VPS10P family sorting receptor Sortilin. *Bioorg Med Chem Lett*. 2014;24(1):177-80.

Tables

Table 1: Clinicopathological characteristics of the breast cancer patient cohort.

Parameter	Randomized untreated (<i>n</i> =232)	Tamoxifen (<i>n</i> =212)	Total (<i>n</i> =444)
Age at diagnosis (year)			
Median	45.00	45.00	45.00
Range	26-57	25-57	25-57
Follow-up time without death from breast cancer			
Median	28.09	28.56	28.41
10 th percentile	20.48	21.35	20.56
90 th percentile	30.85	30.72	30.74
Tumor size (mm)			
Median	23.00	25.00	24.00
Range	2-50	8-75	2-75
Tumor histology			
Ductal	194	176	370
Lobular	18	17	35
Medullar	13	9	22
Missing: 17			
Tumor grade			
Grade 1	31	23	54
Grade 2	100	86	186
Grade 3	96	92	188
Missing: 16			
Lymph node (LN) status			
LN Positive	166	144	310
LN Negative	65	67	132
Missing: 2			
Estrogen receptor (ERa)			
ERa positive	173	144	317

ERα negative	47	49	96
Missing: 31			
Progesterone receptor (PR)			
PR positive	122	108	230
PR negative	63	61	124
Missing: 90			
Human epidermal growth factor receptor 2 (HER2)			
HER2 negative	186	172	358
HER2 positive	35	25	60
Missing: 26			
Progranulin expression			
High	66	73	139
Low	149	124	273
Missing: 32			
Sortilin expression			
High	116	109	225
Low	107	95	202
Missing: 17			

Table 2: Cox regression analysis on randomized untreated patients. Univariate and multivariable interaction analysis on breast cancer-specific survival evaluating various prognostic parameters for relative risk estimation for the untreated patient cohort. HR: hazard ratio, CI: confidence interval, LN: lymph node, ERα: estrogen receptor alpha, HER2: human epidermal growth factor receptor 2.

	Univariate analysis			Multivariable analysis		
Variable	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Grade						
I-II	1			1		
III	1.741	1.210-2.504	0.003	1.737	1.054-2.860	0.030
LN status						
LN negative	1			1		
LN positive	1.964	1.244-3.101	0.004	2.250	1.348-3.758	0.002
Tumor size						
Continuous (mm)	1.014	0.997-1.032	0.108	1.020	0.999-1.041	0.059
Age						
Continuous (per year)	0.966	0.936-0.997	0.033	0.968	0.934-1.003	0.077
ERα						
ERα negative	1			1		
ERα positive	0.824	0.525-1.294	0.401	1.259	0.678-2.340	0.466
HER2						
HER2 negative	1			1		
HER2 positive	1.376	0.849-2.230	0.195	1.152	0.648-2.046	0.629
Progranulin/sortilin combination						
Mixed	1			1		
Double high progranulin/sortilin	1.922	1.224-3.017	0.005	2.188	1.317-3.637	0.003

Figures

Figure 1

Progranulin and sortilin scoring. (A) Representative immunohistochemical images of breast cancer tissue microarray sections showing variations in progranulin and sortilin expression, scored from 1-4, respectively. Brown: positive progranulin/sortilin antibody staining, blue/pale pink: hematoxylin/eosin for nucleus and cytoplasm staining. Scale bar represents 250µm.

Figure 2

Untreated patients with high tumor co-expression of progranulin and sortilin have worse breast cancer-specific survival. (A) Kaplan-Meier curves illustrating breast cancer-specific survival on combined progranulin and sortilin expression, making a double high group (high progranulin, high sortilin expression) against all other combinations (of low/high progranulin/sortilin expression) for the randomized untreated patients only (n=206). (B) BCSS looking at all different progranulin/sortilin combinations in randomized untreated group only (n=206). The statistical differences between the curves, as well as HR and 95% CI were estimated by the log-rank test. BCSS: breast cancer-specific survival, HR: hazard ratio, CI: confidence interval, PGRN: progranulin, SORT: sortilin.

Figure 3

ER α positive patients with high progranulin and sortilin co-expression have worse breast cancer-specific survival. (A-C) Kaplan-Meier curves illustrating breast cancer-specific survival in all ER α positive patients. (A) BCSS in the double high group (high progranulin, high sortilin expression) against all other combinations (mixed group) in the ER α positive population (n=279). (B-C) Tamoxifen response shown by BCSS in all ER α positive patients stratified by (B) double high group (n=55) or (C) mixed progranulin/sortilin expression (n=224). The statistical differences between the curves, as well as HR and 95% CI were estimated by the log-rank test. BCSS: breast cancer-specific survival, HR: hazard ratio, CI: confidence interval, PGRN: progranulin, SORT: sortilin.

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