

RESEARCH

Open Access



Vitamin D receptor, Retinoid X receptor and peroxisome proliferator-activated receptor γ are overexpressed in *BRCA1* mutated breast cancer and predict prognosis

Sabine Heublein^{1,2*}, Doris Mayr³, Alfons Meindl⁴, Alexandra Kircher⁵, Udo Jeschke² and Nina Ditsch²

Abstract

Background: *BRCA1* mutated breast cancers are commonly diagnosed as negative for classical hormone receptors i.e. estrogen receptor, progesterone receptor and/or Her2. Due to these common targets being absent the application of anti-endocrine therapies is rather limited and a certain focus has been set on discovering alternative target molecules. We recently highlighted thyroid hormone receptors (TRs) to predict prognosis in breast cancer patients that had been diagnosed a *BRCA1* germline mutation. Vitamin D Receptor (VDR), Retinoid X Receptor (RXR) and Peroxisome Proliferator-activated Receptor γ (PPAR γ) are known to interact with TRs by forming functional heterodimers. Whether VDR, RXR or PPAR γ are expressed in *BRCA1* mutated breast cancer or may even be present in case of triple negativity is not known. Hence the current study aimed to investigate VDR, RXR and PPAR γ in *BRCA1*^{mut} breast cancer and to test whether any of the three may be associated with clinico-pathological criteria including overall survival.

Methods: This study analyzed VDR, RXR and PPAR γ by immunohistochemistry in *BRCA1* associated ($n = 38$) and sporadic breast cancer ($n = 79$). Receptors were quantified by applying an established scoring system (IR-score) and were tested for association with clinico-pathological variables.

Results: VDR, RXR and PPAR γ were detected in over 90% of triple negative *BRCA1*^{mut} breast cancer and were significantly (VDR: $p < 0.001$, RXR: $p = 0.010$, PPAR γ : $p < 0.001$) overexpressed in *BRCA1* mutated as compared to sporadic cancer cases. VDR and RXR positivity predicted prolonged overall survival only in *BRCA1* mutated cases while such association was not observed in sporadic breast cancer.

Conclusions: In conclusion, this is the first study to describe VDR, RXR and PPAR γ in *BRCA1* mutated breast cancer. Based on the data presented here these receptors may be hypothesized to potentially evolve as interesting markers or even targets in hereditary breast cancer. However, independent studies are indispensable thus to confirm this hypothesis.

Keywords: Vitamin D Receptor (VDR), Retinoid X Receptor (RXR), Peroxisome-proliferator activated Receptor γ (PPAR γ), Hereditary breast cancer, Survival, *BRCA1*

* Correspondence: sabine.heublein@med.uni-heidelberg.de

¹Department of Obstetrics and Gynaecology - National Center for Tumor Diseases (NCT), Heidelberg University Hospital, Heidelberg, Germany

²Department of Obstetrics and Gynecology, Ludwig-Maximilians-University of Munich, Munich, Germany

Full list of author information is available at the end of the article



Background

Immunoreactivity of estrogen receptor (ER), progesterone receptor (PR) or Her2 in breast cancer tissue provides both predictive and prognostic information. Determination of ER, PR and Her2 has evolved to be mandatory during routine clinical management of breast cancer patients. In general, about two third of breast cancer cases are regarded to express at least ER on immunohistochemistry level [1, 2] making them particularly predestined for anti-endocrine treatment options. On the other hand treatment of those breast cancers being negative for ER, PR or Her2 is considered to be rather challenging. At least partly this may also be caused by the fact that due to the lack of hormone receptors anti-endocrine treatments options do not apply. Hence this group of ER negative or even triple negative (i.e. negative for ER, PR and Her2) breast cancers has attracted extensive attention during the last years [3]. While treatment options in Her2 positive breast cancer have notably improved since the approval of Her2 targeted agents [4], clinical management of triple negative breast cancer remains to be rather challenging [5]. Especially those patients that have been identified to carry a germ line mutation in *BRCA1* are diagnosed as negative for ER, PR or Her2 to an unusually high extend [6]. Since the frequent lack of hormone receptors (ER/PR) or Her2 extensively narrows the application of (anti-) endocrine therapies, *BRCA1* associated breast cancers require a specially tailored therapeutic regimen [7]. As a consequence the search for alternative predictive/prognostic biomarkers is on the increase.

Like ER or PR, which have also been referred to as 'classical steroid hormone receptors', Vitamin D Receptor (VDR), Retinoid X Receptor (RXR), Peroxisome Proliferator-activated Receptor γ (PPAR γ) and Thyroid Hormone Receptors (TRs) are members of the nuclear hormone receptor superfamily. There are several lines of evidence that VDR, RXR or PPAR γ may be of relevance in breast cancer tumorigenesis. First, TRs are known to assemble with VDR, RXR and PPAR γ by forming functional heterodimers. We recently highlighted thyroid hormone receptors (TRs) to be widely expressed in breast cancer tissue deriving from patients diagnosed with a *BRCA1* germline mutation. TRs were of opposing prognostic significance and silencing of TR α appeared to diminish viability of *BRCA1* mutated breast cancer cells [8]. Further, polymorphisms in VDR have been demonstrated to be associated with breast cancer risk [9] and both RXR and PPAR γ have been demonstrated to comprise anti-cancer cell activity [10–12].

However, neither VDR nor RXR nor PPAR γ have been studied in *BRCA1* associated breast cancer so far. As VDR, RXR and PPAR γ can be quantified in cancer tissue easily, they - given them being present in *BRCA1* mutated breast cancer cases at all - may evolve as novel alternative

biomarkers, especially for hormone receptor negative or even triple negative breast cancer patients.

Methods

Aim of the study

Aim of the current study was to investigate whether VDR, RXR and PPAR γ in *BRCA1*^{mut} are expressed in breast cancer tissue and whether any of the three may be associated with clinico-pathological criteria including overall survival.

Patients

One hundred twenty-four patients diagnosed with sporadic ($n = 86$) or *BRCA1* associated cancer ($n = 38$) of the breast were analyzed retrospectively in the current study. Patients had undergone breast cancer surgery at the Department of Obstetrics and Gynecology of the Ludwig-Maximilians-University of Munich, Germany between 1987 and 2009. Following resection breast cancer tissue underwent formalin fixation and paraffin embedding. Formalin fixed paraffin embedded (FFPE) tissue used in this study had been stored under standardized conditions. Thyroid hormone receptor profiling on the same patient panel has already been published [8]. Seven cases were no longer available and hence VDR, RXR and PPAR γ staining was only performed in 79 sporadic and 38 *BRCA1*^{mut} samples. Except from correlation analysis (Table 4), calculations were done on this slightly reduce panel on which VDR, RXR and PPAR γ data were available ($n = 117$). Breast cancer of non-specific type (NST) was diagnosed in 91 (91/117, 77.8%) of the patients and 62.1% (72/116) were graded as high grade (G3). A significant fraction of the study sample was staged higher than pT1 ($n = 73$, 62.4%) or presented with lymph node ($n = 62$, 56.4%) or distant metastasis ($n = 49$, 46.2%) at time of initial diagnosis. Sufficient information thus to conclude on breast cancer subtypes (Luminal A ($n = 15$), Luminal B ($n = 14$), Her2 positive ($n = 25$), triple negative ($n = 18$)) was available in 72 cases. Mean age (\pm STDV) of the cohort was 49.8 ± 13.4 years (*BRCA1* associated cases: 41.9 ± 10.8 years; sporadic breast cancer: 53.6 ± 12.9 years). See Table 1 for further details.

Study design

Patients' data used within the current study were retrieved from patients' charts, from the Munich Cancer Registry and by direct contact in a retrospective manner. Benign tumors of the breast or patients diagnosed for *in situ* carcinoma were excluded. The outcome assessed was patients' overall survival. Overall mean survival of the cohort was 7.31 years (95% CI: 6.24 - 8.38 years) and mean follow up time was 6.82 years (95% CI: 5.90 - 7.75 years). Mean follow up time for all patients still alive at time of analysis was 5.26 years.

Table 1 Patient characteristics, whole study panel

[IRS]		<i>BRCA1</i> ^{mut}		<i>p</i>	<i>BRCA1</i> ^{mut}									sporadic								
			sporad.		VDR			RXR			PPAR γ			VDR			RXR			PPAR γ		
					<4	\geq 4	<i>p</i>	<4	\geq 4	<i>p</i>	<4	\geq 4	<i>p</i>	<4	\geq 4	<i>p</i>	<4	\geq 4	<i>p</i>	<4	\geq 4	<i>p</i>
suptype	other	8	18	ns	3	5	ns	1	7	ns	4	2	ns	11	6	ns	11	7	ns	16	2	ns
	NST	30	61		4	25		11	19		12	8		40	21		30	31		55	6	
G	G1-2	8	36	0.015	2	5	ns	2	6	ns	4	3	ns	30	6	0.004	17	19	ns	35	1	ns
	G3	29	43		5	24		10	19		12	7		21	21		24	19		36	7	
pT	pT1a-c	21	23	0.008	3	17	ns	4	17	ns	9	5	ns	14	9	ns	9	14	ns	19	4	ns
	pT2-pT4	17	56		4	13		8	9		7	5		37	18		32	24		52	4	
pN	pN0	20	28	ns	2	18	ns	4	16	ns	9	5	ns	16	11	ns	14	14	ns	22	6	0.046
	pN+	15	47		5	10		7	8		6	4		31	16		23	24		45	2	
pM	pM0	18	21	ns	4	13	ns	4	14	ns	5	6	ns	8	12	0.031	8	13	ns	18	3	ns
	pM+	18	49		3	15		8	10		10	4		34	15		27	22		44	5	
ER	negative	27	24	0.002	6	20	ns	8	19	ns	9	9	ns	12	11	ns	15	9	ns	18	6	0.044
	positive	11	40		1	10		4	7		7	1		28	12		20	20		38	2	
PR	negative	27	23	0.001	4	22	ns	8	19	ns	9	9	ns	14	8	ns	15	8	ns	18	5	ns
	positive	11	41		3	8		4	7		7	1		26	15		20	21		38	3	
Her2	negative	22	25	ns	3	18	ns	5	17	ns	9	8	ns	17	7	ns	12	13	ns	22	3	ns
	positive	6	19		1	5		3	3		4	1		9	10		9	10		17	2	
Triple-negative	no	17	51	0.002	4	13	ns	7	10	ns	11	2	0.007	32	19	ns	25	26	ns	48	3	0.012
	yes	12	6		0	11		2	10		2	7		4	1		3	3		3	3	
Ki67	low	10	50	ns	4	5	ns	4	6	ns	6	4	ns	32	18	ns	29	21	ns	45	5	ns
	high	10	20		2	8		4	6		6	3		13	6		9	11		17	3	
age	\leq 41.5 y	19	16	0.002	3	15	ns	4	15	ns	4	6	ns	8	8	ns	6	10	ns	15	1	ns
	>41.5 y	19	62		4	15		8	11		12	4		42	19		34	28		55	7	

ns not significant

Assay methods

Mutation screening

Mutation analysis was described by Fischer et al. [13] and was performed at a German center for *BRCA1* mutation testing (Technical University of Munich, Munich, Germany) according to a standardized protocol. Briefly, high performance liquid chromatography (dHPLC) and sequencing of conspicuous amplicons was employed thus to analyze PCR products comprising all coding exons of *BRCA1*. Alternatively, direct sequencing of all *BRCA1* amplicons was performed. The NCBI (National Center for Biotechnology Information) cDNA sequence U14680.1 (*BRCA1*) served as a reference. In case of a negative sequencing results multiplex ligation-dependent probe amplification (MLPA) was used to screen for deletions or duplications in *BRCA1*. Variants of unknown significance (VUS) characterized as VUS Class III were not considered as mutations.

Immunostaining

Immunohistochemistry of VDR, RXR and PPAR γ on FFPE sections had been described by our group [14, 15]. In

brief, antibodies detecting VDR (mouse anti human VDR, monoclonal, AbD Serotec, Oxford, UK), RXR (mouse anti human RXR, monoclonal, Perseus Proteomics Inc., Tokyo, Japan) and PPAR γ (rabbit anti human RXR, polyclonal, Abcam, Cambridge, MA, USA) were stained by employing standardized procedures and commercially available kits (Vectastain Elite mouse-IgG-Kit for VDR, RXR staining; ZytoChem Plus HRP Polymer System (Mouse/Rabbit) for detection of PPAR γ). Placenta tissue which had been demonstrated to express VDR, RXR as well as PPAR γ served as positive control [14]. Placenta and breast cancer sections treated with pre-immune rabbit IgG (supersensitive rabbit negative control, BioGenex, Fremont, CA) or isotype matched mouse IgGs (Dako, Hamburg, D) instead of the primary antibody were used as negative controls. Positive and negative controls were included in each experiment. A well-established semiquantitative scoring system (IR-score or Remmele score) was employed thus to quantify immunostaining in a semi-quantitative manner [14, 16–18]. Scoring was performed by two independent observers by consensus. This scoring method has already been proven suitable to assess VDR, RXR and

PPAR γ immunostaining [15–17]. The IR-score quantifies immunoreactivity by multiplication of staining intensity (graded as 0: none, 1: weak, 2: moderate and 3: strong staining) and percentage of positively stained cells (0: no staining, 1: \leq 10% of the cells, 2: 11–50% of the cells, 3: 51–80% of the cells and 4: \geq 81% of the cells). Tissue samples that had been assigned an IR-Score higher or equal to IRS 4 (i.e. IRS 4, IRS 6, IRS 8, IRS 9, IRS 12) were scored as positive. This cut-off was set based on mean expression of VDR (mean IRS [$n = 117$] = 4.14) and RXR (mean IRS [$n = 117$] = 4.28).

Assessment of Her2 has been described in [19]. Her2 staining of cases that had been diagnosed before routine HER2 staining was performed, was done on archived FFPE samples where available. Thus to conclude on breast cancer subtypes, we also performed Ki67 staining. Samples were stained using an anti-Ki67 monoclonal antibody (Dako, Hamburg, Germany) at a dilution of 1:150 on a VENTANA®-Benchmark Unit (Roche, Mannheim, Germany). Scoring was performed according to our local standards also applying for routine clinical diagnostics: score 0 = 0% positive cells, score 1: < 5% positive cells, score 2: < 10% positive cells, score 3: < 20% positive cells, score 4: > 20% positive cells). Tissue samples that had been assigned a Ki67-Score higher than score 2 (i.e. score 3 or 4) were scored as positive. Overexpression of Ki67 was defined as Ki67-Score 4.

Statistical analysis methods

This study has been carried out according to the REMARK (Reporting Recommendations for Tumor Marker Prognostic Studies) criteria [20]. The IBM statistic package SPSS (version 23) was used to test data for statistical significance. Fisher's exact test and the Mann–Whitney test were used thus to test differences for statistical significance. Survival times were compared by Kaplan–Meier analysis and

differences in patient overall survival times were tested for significance by using the chi-square statistics of the log rank test.

Statistical analysis were also done in a group of 54 patients (n (sporadic) = 27, n (*BRCA1* associated) = 27). These patients had been matched ($p = 1.000$) according to tumor size, lymph node status, presence of metastasis and tumor grade.

Results

Study cohort

There was no significant difference in terms of histologic subtype, presence of lymph node or distant metastasis or Her2 positivity when sporadic cancer cases and those carrying a *BRCA1* germline mutation were compared. *BRCA1*^{mut} cases were smaller in size ($p = 0.008$), less well differentiated ($p = 0.015$) and more often classified as negative for 'classical' hormone receptors (ER: $p = 0.002$; PR: $p = 0.001$) as compared to sporadic cancers. In line with this, *BRCA1*^{mut} cancers were more often found to be triple negative ($p = 0.002$). Finally, *BRCA1*^{mut} patients were significantly younger ($p = 0.002$) at the time of primary diagnosis (Table 1).

VDR, RXR and PPAR γ are overexpressed in *BRCA1*^{mut} breast cancer cases

VDR and RXR were expressed with a prominent nuclear pattern, while PPAR γ staining was mainly located in the cytoplasm (Fig. 1). In case of all the three antigens staining was found restricted to cancer cells whereas stroma tissue and intercellular spaces stained negative. Regarding the total patient sample both VDR and RXR were expressed in about half of all cases (VDR: 57/115, 49.6%; RXR: 64/117, 54.7%) while much less cases were detected to stain positive for PPAR γ (PPAR γ : 18/105, 17.1%). Apart from PPAR γ overexpression in triple negative cancers ($p = 0.007$) no

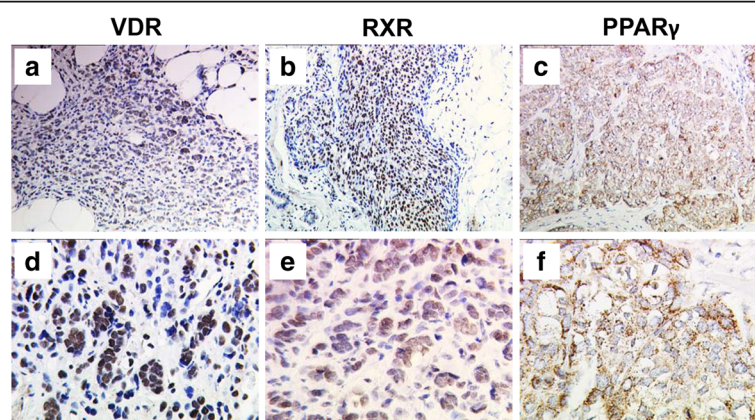


Fig. 1 VDR, RXR and PPAR γ immunostaining in *BRCA1* mutated breast cancer. VDR, RXR and PPAR γ were detected by immunohistochemistry in *BRCA1* mutated breast cancer. Representative images are shown; magnification: **a-c**: 10 \times lens, **d-f**: 25 \times lens

correlation of clinico-pathological parameters and VDR, RXR or PPAR γ positivity was detected in *BRCA1*^{mut} patients (Table 1). In terms of sporadic cancer VDR was overexpressed in poorly differentiated ($p = 0.004$) or non-metastasized (M0, $p = 0.031$) cases. PPAR γ was negatively correlated with lymph node involvement ($p = 0.046$) and ER staining ($p = 0.044$). Interestingly, PPAR γ ($p = 0.012$) was expressed in half of those sporadic cases that were diagnosed as triple negative. No association of RXR and clinico-pathological variables was detected in terms of sporadic breast cancer. Patients carrying a *BRCA1* germline mutation overexpressed VDR (*BRCA1*^{mut} vs. sporadic: IRS 6.00 vs. IRS 3.00%, $p < 0.001$), RXR (*BRCA1*^{mut} vs. sporadic: IRS 6.00 vs. IRS 3.00, $p = 0.010$) and PPAR γ (*BRCA1*^{mut} vs. sporadic: IRS 2.00 vs. IRS 0.00, $p < 0.001$) when compared to sporadic breast cancer (Fig. 2). A similar effect could be reproduced when sporadic and *BRCA1*^{mut} cancers were compared with respect to breast cancer subtypes (Table 2).

BRCA1 mutation has been associated with the basal like breast cancer subtype [21]. Among other criteria loss of hormone receptors, loss of Her2, positivity for Ki67 and poor differentiation have been identified to be characteristics of basal like breast cancers [22, 23]. Our patient sample contained 18 TNBC cases with 14 of them being classified as high grade. Information on Ki67 was available in 13 of 18 TNBC cases. Information on both ki67 and Grading was available in 13 cases. Seven of these 13 TNBC samples were identified as both high grade and overexpressing Ki67, therefore comprising at least 'basic basal like' features. Interestingly, 5 out of 7 (71%) *BRCA1*^{mut} cases but only 2 out of 6 (33%) sporadic TNBC cases showed highly proliferative i.e. 'basic basal like' characteristics as explained above (Additional file 1: Figure S1). VDR, RXR and PPAR γ were expressed in 5 (VDR), 4

(RXR) and 3 (PPAR γ) out of 5 highly proliferative *BRCA1*^{mut} TNBC cases.

BRCA1^{mut} breast cancer is commonly found to be negative for classical hormone receptors i.e. ER, PR and/or Her2. We then analyzed expression of VDR, RXR and PPAR γ in those *BRCA1*^{mut} cases that stained negative for classical hormone receptors. Interestingly, the vast majority of hormone receptor negative, *BRCA1*^{mut} cases were positive for VDR, RXR or PPAR γ . In particular, triple negative breast cancer stained positive in up to 100% of the cases (Fig. 3).

Comparisons were repeated in the matched patient panel (Table 3) and overexpression of nuclear receptors in *BRCA1*^{mut} as compared to sporadic cancer cases could be reproduced in terms of VDR (*BRCA1*^{mut} vs. sporadic: IRS 6.00 vs. IRS 3.50 $p = 0.008$) as well as PPAR γ (*BRCA1*^{mut} vs. sporadic: IRS 6.00 vs. IRS 4.00, $p = 0.008$). Further, PPAR γ remained to be overexpressed in triple negative cancers regarding both *BRCA1*^{mut} ($p = 0.036$) and sporadic ($p = 0.040$) cases. Further, PPAR γ was positively correlated to pN0 ($p = 0.047$) in sporadic cancers. A positive correlation of RXR and PR ($p = 0.008$) was observed. Finally, RXR and VDR were found to be inversely correlated to grading (RXR - grading: $p = 0.022$) or presence of distant metastasis (VDR - pM: $p = 0.041$).

Correlation analysis

Nuclear hormone receptors are known to act as heterodimers. Thus to conclude on potential heterodimerization correlation analysis was performed. Several correlations among VDR, RXR and PPAR γ among each other were observed both in *BRCA1*^{mut} and sporadic cases (Table 4). Interestingly, RXR (*BRCA1*^{mut}: $p = 0.014$) and PPAR γ (sporadic: $p = 0.035$) immunoreactivity was found to rise

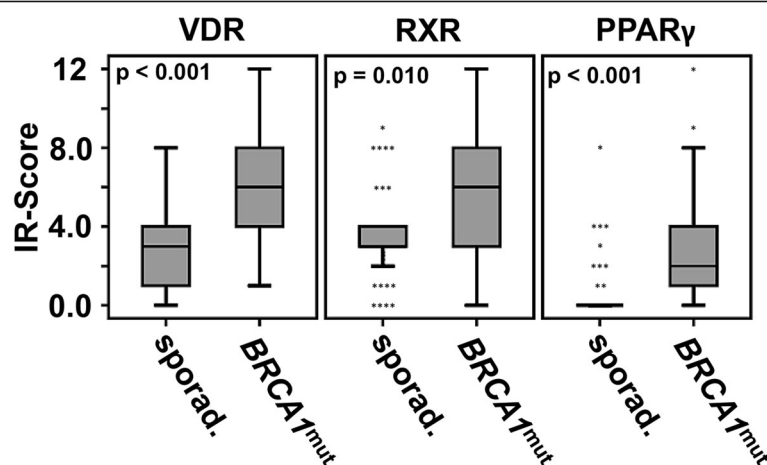


Fig. 2 VDR, RXR and PPAR γ are significantly up-regulated in *BRCA1* mutated breast cancer tissue. VDR, RXR and PPAR γ immunoreactivity of sporadic vs. *BRCA1* mutated breast cancer tissue was compared. All the three receptors were found to be significantly up-regulated in a *BRCA1* mutated genetic background. *P*-values derived from relevant Mann-Whitney-U tests are shown

Table 2 VDR, RXR and PPAR γ immunopositivity in breast cancer subgroups

	VDR (median IRS)			RXR (median IRS)			PPAR γ (median IRS)		
	<i>BRCA1</i> ^{mut}	sporadic	<i>p</i>	<i>BRCA1</i> ^{mut}	sporadic	<i>p</i>	<i>BRCA1</i> ^{mut}	sporadic	<i>p</i>
Luminal A	3,5	1,0	0,142	6,0	4,0	0,223	1,5	0,0	0,002
Luminal B	8,0	3,5	0,026	6,0	3,5	0,23	1,0	0,0	0,032
Her2 positive	8,0	4,0	0,023	4,5	4,0	0,769	1,0	0,0	0,061
TNBC	8,0	3,0	0,003	8,0	2,5	0,036	6,0	2,0	0,072

Median VDR, RXR and PPAR γ immunopositivity was analyzed in breast cancer subtypes.

in parallel to TR β positivity, while no correlation to oncogenic TR α was observed.

Absence of VDR or RXR is associated with reduced overall survival in *BRCA1*^{mut} cases

VDR, RXR and PPAR γ were correlated to patients' overall survival (OS). Studying the whole patient sample OS of those cases that stained negative for either VDR (95% CI (VDR negative): 4.43 y - 7.14 y; 95% CI (VDR positive): 7.39 y - 10.7 y; *p* = 0.004, Fig. 4a) or RXR (95% CI (RXR negative): 4.37 y - 7.47 y; 95% CI (RXR positive): 6.90 y - 9.72 y; *p* = 0.009, Fig. 4b) was found to be significantly reduced. However, no statistical association to OS was detected in case of sporadic cancer cases (Fig. 4g-i). Negativity for either VDR (95% CI (VDR negative): 0.69 y - 9.38 y; 95% CI (VDR positive): 10.3 y - 13.4 y; *p* = 0.019, Fig. 4d) or RXR (95% CI (RXR negative): 3.92 y - 11.3 y; 95% CI (RXR positive): 10.1 y - 12.2 y; *p* = 0.007, Fig. 4e) was associated with significantly shortened overall survival in those cases that had been diagnosed a *BRCA1* germline mutation. PPAR γ was not related to OS at all (Fig 4c, f, i).

Thus to avoid confounding effects survival analysis were repeated in those patients that had been matched for clinico-pathological variables. Interestingly, both VDR (95% CI (VDR negative): 2.47 y - 5.67 y; 95% CI (VDR positive): 9.61 y - 12.9 y; *p* < 0.001, Fig. 5a) and RXR (95% CI (RXR negative): 3.23 y - 8.52 y; 95% CI (RXR positive): 6.80 y - 10.8 y; *p* = 0.005, Fig. 5b) positivity predicted favorable OS in the matched patient panel prior to stratification.

Following stratification this only remained to be significant in those patients that had been diagnosed a germline mutation in *BRCA1* (95% CI (VDR negative): 0.69 y - 9.38 y; 95% CI (VDR positive): 9.98 y - 13.7 y; *p* = 0.047, Fig. 5d; 95% CI (RXR negative): 2.98 y - 10.8 y; 95% CI (RXR positive): 10.1 y - 12.6 y; *p* = 0.008, Fig. 5e). Again, no association of PPAR γ and OS was observed (Fig. 5c, f, i).

Discussion

VDR, RXR and PPAR γ overexpression in *BRCA1*^{mut} breast cancer

To the best of our knowledge this analysis is among the first to show immunopositivity of VDR, RXR and PPAR γ in *BRCA1*^{mut} breast cancer patients. There is some evidence that absence of functional BRCA may alter expression of these so called 'thyroid hormone receptor like genes'. For instance, knockdown of *BRCA2* in breast cancer cell lines modulated expression of RXR isoforms in opposing ways and knockout of *BRCA1* reduced expression of PPAR γ in cardiomyocytes [24, 25]. Some authors even reported a physical interaction of VDR and BRCA1 protein [26, 27]. Hence, loss of functional *BRCA1* may lead to compensatory up-regulation of VDR. Whether a scenario like this may explain overexpression of VDR in *BRCA1*^{mut} breast cancer cases remains to be elucidated. E3 Ubiquitin ligase activity of functional BRCA1 may serve as an alternative way to interpret overexpression of thyroid like receptors in a *BRCA1* mutant genetic background. Wildtype BRCA1 protein contributes to degradation of nuclear hormone receptors

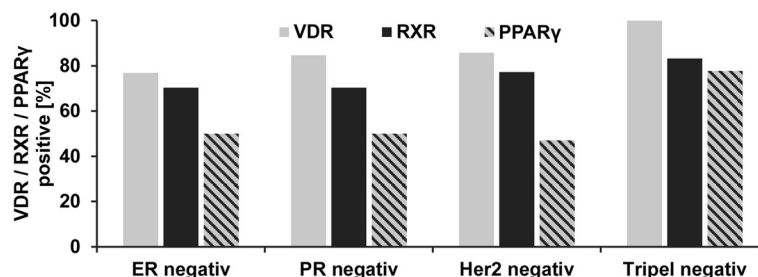


Fig. 3 Percentages of VDR, RXR and PPAR γ cases in respect to hormone receptor expression. VDR, RXR and PPAR γ positive cases in ER negative, PR negative, Her2 negative and triple negative cancer were counted and are displayed as percentage in respect to the respective hormone receptor profile. Even in absence of classical hormone receptors VDR, RXR or PPAR γ were detected in the large majority of cases

Table 3 Patient characteristics, matched groups

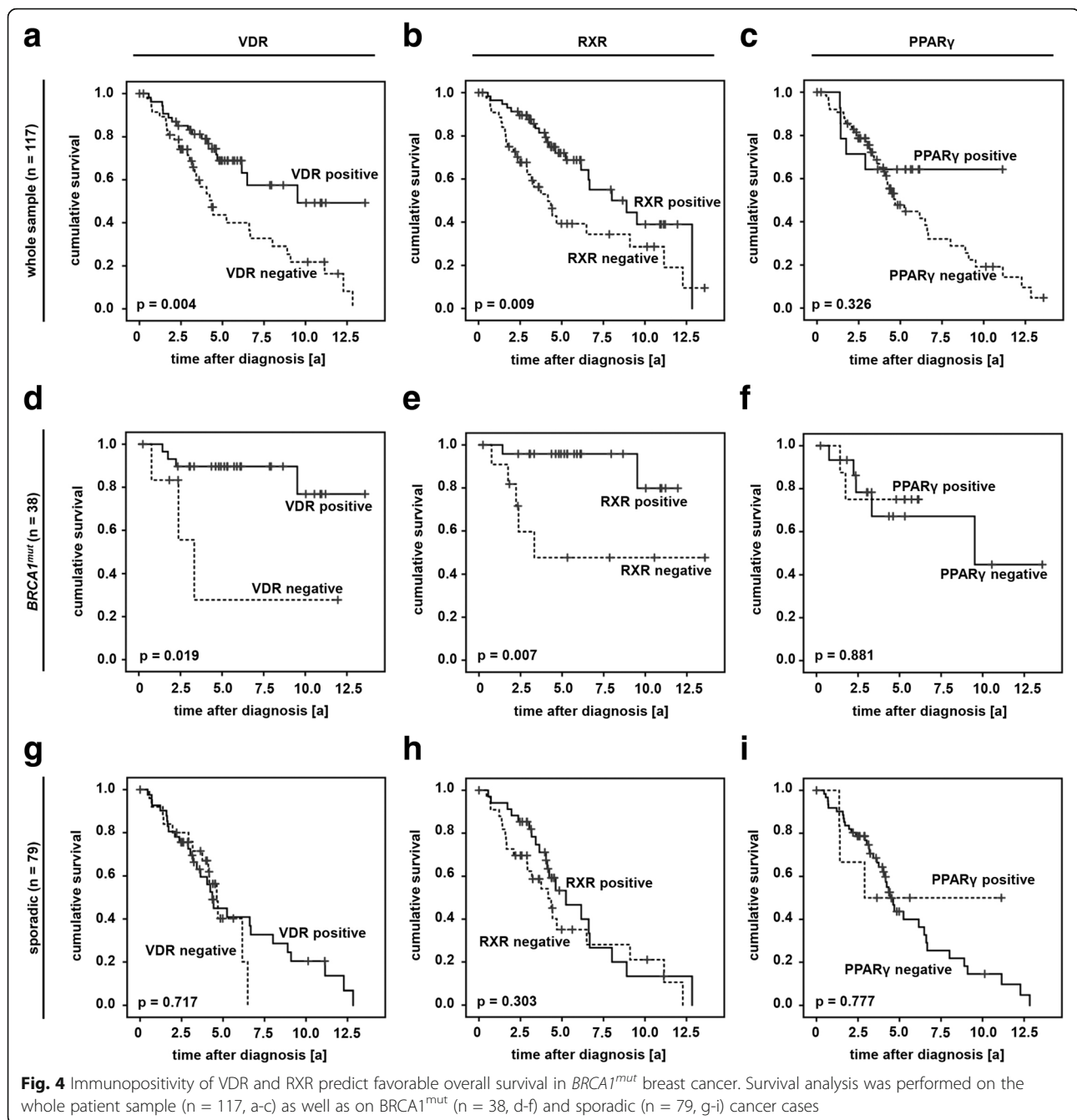
[IRS]		<i>BRCA1</i> ^{mut}									sporadic								
		VDR			RXR			PPAR γ			VDR			RXR			PPAR γ		
		<4	\geq 4	<i>p</i>	<4	\geq 4	<i>p</i>	<4	\geq 4	<i>p</i>	<4	\geq 4	<i>p</i>	<4	\geq 4	<i>p</i>	<4	\geq 4	<i>p</i>
suptype	other	3	2	ns	1	4	ns	4	0	ns	2	3	ns	3	3	ns	5	1	ns
	NST	4	18		9	13		9	7		11	10		8	13		17	4	
G	G1-2	2	5	ns	2	5	ns	4	2	ns	5	2	ns	0	7	0.022	7	0	ns
	G3	5	15		8	12		9	5		8	11		11	9		15	5	
pT	pT1a-c	3	9	ns	3	9	ns	8	2	ns	4	8	ns	5	7	ns	9	3	ns
	pT2-pT4	4	11		7	8		5	5		9	5		6	9		13	2	
pN	pN0	2	13	ns	3	12	ns	8	4	ns	6	8	ns	7	8	ns	10	5	0.047
	pN+	5	7		7	5		5	3		7	5		4	8		12	0	
pM	pM0	4	7	ns	3	8	ns	4	3	ns	2	8	0.041	3	8	ns	10	1	ns
	pM+	3	13		7	9		9	4		11	5		8	8		12	4	
ER	negative	6	13	ns	7	12	ns	8	6	ns	8	8	ns	9	8	ns	12	5	ns
	positive	1	7		3	5		5	1		5	5		2	8		10	0	
PR	negative	4	16	ns	7	13	ns	8	6	ns	9	6	ns	10	6	0.008	12	4	ns
	positive	3	4		3	4		5	1		4	7		1	10		10	1	
Her2	negative	3	13	ns	4	12	ns	8	5	ns	7	4	ns	4	8	ns	9	3	ns
	positive	1	3		2	2		2	1		2	7		2	7		8	1	
Triple-negative	no	4	9	ns	6	7	ns	9	2	0.036	7	10	ns	4	13	ns	16	1	0.040
	yes	0	8		1	7		1	4		4	1		3	3		3	3	
Ki67	low	5	5	ns	6	4	ns	7	3	ns	8	9	ns	8	9	ns	13	4	ns
	high	1	4		1	4		3	1		4	2		3	4		6	1	
age	\leq 41.5 y	3	9	ns	4	8	ns	3	4	ns	1	3	ns	1	3	ns	4	0	ns
	>41.5 y	4	11		6	9		10	3		12	10		10	13		18	5	

ns not significant

Table 4 Correlation analysis

			<i>BRCA1</i> ^{mut}					sporadic				
			VDR	RXR	PPAR γ	TR α	TR β	VDR	RXR	PPAR γ	TR α	TR β
Spearman's rho	VDR	cc	1.000	0.336	0.538	-.008	.191	1.000	.042	0.256	.028	.097
		Sig.	x	.042*	.005*	.964	.257	x	.718	.024*	.810	.397
		n	37	37	25	37	37	78	78	78	78	78
	RXR	cc		1.000	0.578	-.172	0.395		1.000	-.0325	-.031	.045
		Sig.		x	.002*	.302	.014*	x	.003*	.786	.693	
		n		38	26	38	38	79	79	79	79	
	PPAR γ	cc			1.000	-.001	.241			1.000	-.063	0.237
		Sig.			x	.997	.236	x		.582	.035*	
		n			26	26	26		79	79	79	
TR α	cc				1.000	-.192				1.000	.195	
	Sig.				x	.248				x	.071	
	n				38	38				86	86	
TR β	cc					1.000					1.000	
	Sig.					x					x	
	n					38					86	

Correlation analysis was performed in *BRCA1*^{mut} as well as in sporadic cases. Significant correlations are indicated by asterisks (*: *p* < 0.05)

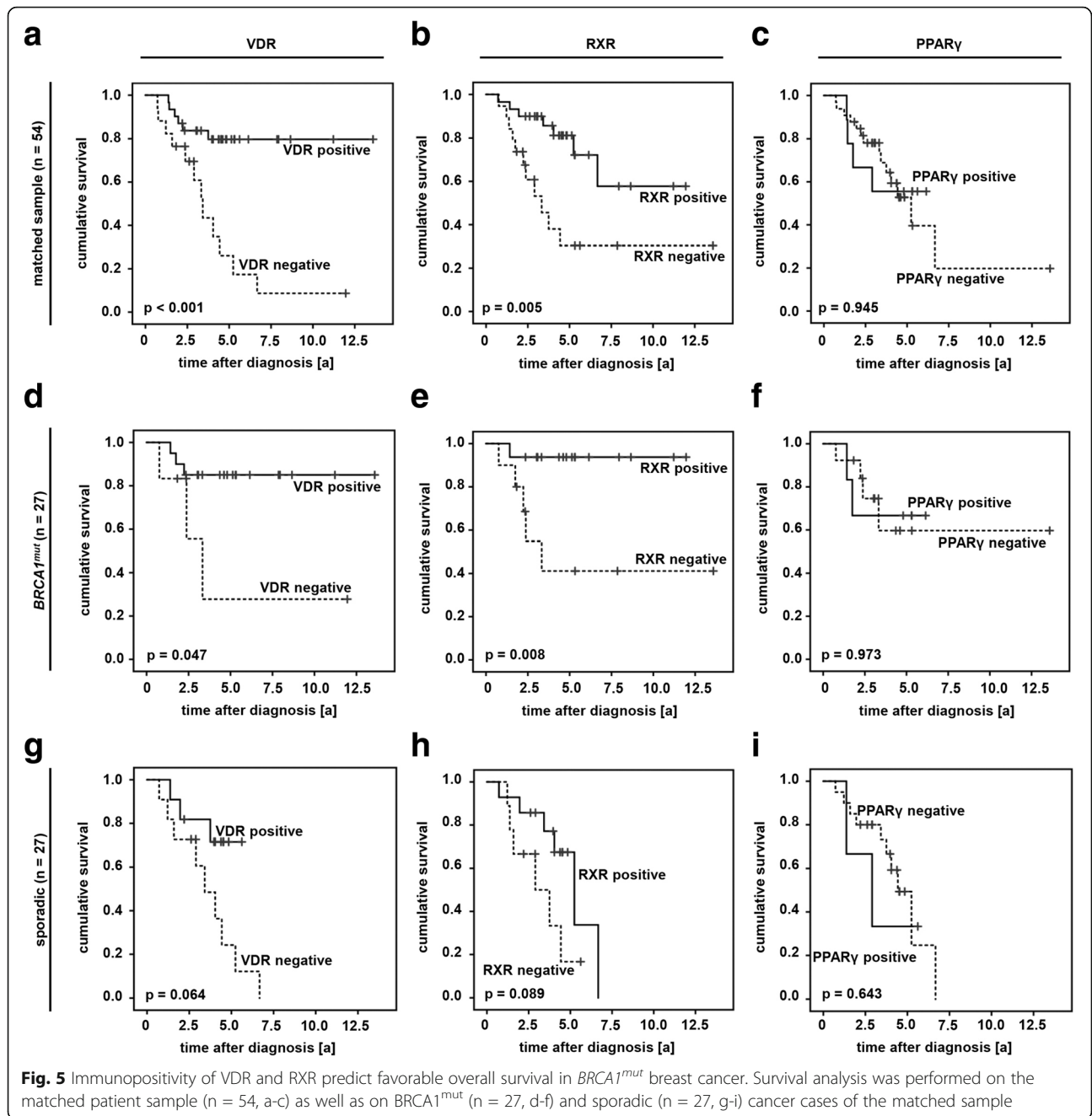


including VDR, RXR and PPAR γ via its ubiquitination and sumoylation activity [28–31]. Thus loss of functional *BRCA1* may explain overexpression of these receptors in *BRCA1* mutant genetic background. A similar mechanism was demonstrated in case of thyroid hormone receptors in *BRCA1^{mut}* breast cancer [8]. Besides carrying a *BRCA* mutation, cancers expressing alternative nuclear hormone receptors were often found to be triple negative. VDR, RXR and PPAR γ were even detected in those cancers characterized as both triple negative and highly proliferative - two basic features of basal like breast cancer [22, 23].

Comprehensive analysis of “basal like biomarkers” performed in a larger patient sample is mandatory thus to properly select basal like breast cancer cases and to further study the role of alternative hormone receptors in basal like breast cancer.

VDR and RXR in *BRCA1^{mut}* breast cancer - translational aspects

Though several significant associations of VDR, RXR and PPAR γ to clinico-pathological variables were observed in sporadic breast cancer, there was only one significant



correlation detected when VDR, RXR and PPAR γ were correlated to clinico-pathological variables in *BRCA1^{mut}* cases. A comprehensive study on VDR in 1116 breast cancer patients revealed VDR to correlate to those clinic-pathological variables that may indicate lower tumor-biologic aggressiveness [32]. We found that presence of VDR is positively correlated to the absence of distant metastasis. In line with others [15, 33] this suggests that VDR expression may exert differentiating effects on breast cancer cells. This is in agreement with the fact that absence of VDR was correlated with shortened OS of patients carrying a *BRCA1*

mutation in the current analysis. However results on whether VDR may predict prognosis in breast cancer are not consistent throughout the literature. While a former study of our group detected VDR to correlate with favorable OS [16], others did not find an association of VDR and breast cancer prognosis [32]. So far, no data on the prognostic significance of VDR in *BRCA1* mutated breast cancer have been published. Whether the association of VDR and OS observed in the current work may be enhanced due to the absence of wildtype *BRCA1* remains to be validated in larger trials as well as on a functional level.

Strikingly, Thakkar et al. recently highlighted that VDR agonists may inhibit proliferation of triple negative, VDR positive breast cancer cell lines in a receptor dependent manner [34]. This observation further supports a tumor suppressor like activity of VDR and may further strengthen a potential role of VDR as a promising new target in triple negative breast cancer.

There is some evidence that activation of RXR may induce apoptosis in breast cancer cells and may reduce cell growth [35, 36]. Even less is known on how RXR may affect tumor-biologic characteristics on *BRCA1^{mut}* breast cancer and no data on prognostic significance of RXR in hereditary breast cancer have been published so far. Our results presented suggest that RXR positivity may predict favorable prognosis in breast cancer. Whether *BRCA1^{mut}* breast cancer may be sensitive to RXR modulating drugs and whether this may affect tumor biology or even clinical outcome remains to be determined - with the same applying for VDR, respectively.

Conclusion

The present study demonstrated *BRCA1^{mut}* breast cancer cases to overexpress VDR, RXR and PPAR γ - especially in the absence of 'classical' hormone receptors. Further loss of both VDR and RXR predicted shortened overall survival in *BRCA1^{mut}* breast cancer. Therefore VDR may act as a tumor suppressor in presence of *BRCA1^{mut}* and may potentially evolve as a promising new target in the future.

Additional file

Additional file 1: Figure S1. Highly proliferative cancers within TNBC. (JPG 150 kb)

Abbreviations

FFPE: Formalin fixed paraffin embedded; IRS: Immuno-reactive score; ns: Not significant; PPAR γ : Peroxisome Proliferator-activated Receptor γ ; RXR: Retinoid X Receptor; TRs: Thyroid hormone receptors; VDR: Vitamin D Receptor

Acknowledgements

The authors would like to thank Christina Kuhn, Susanne Kunze (†) and Irmgard Wiest for their excellent technical assistance. We thank Professor Jutta Engel (Munich Cancer Registry) and Verena Lanser for their help with retrieving follow-up data. The funder had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. "This work was honored with the Poster Award 2015 of the German Breast Group (GBG)".

Funding

This work was supported by a Deutschlandstipendium scholarship of the Ludwig-Maximilians-University of Munich Medical Faculty to SH. This work was awarded with the 2015 poster prize of the German Breast Group (GBG) to SH.

Availability of data and materials

All data generated or analysed during this study are included in this published article (and its supplementary information file).

Authors' contributions

SH: Participated in design and coordination of the study, participated in immunohistochemistry assays and analysis, performed the statistical analysis and wrote the manuscript. DM: supervised immunohistochemistry as a gynecologic pathologist and participated in immunohistochemistry analysis,

participated in the design and coordination of the study. AM: carefully read the manuscript for important intellectual content, participated in the design of the study. AK: carefully read the manuscript for important intellectual content. UJ: conceived of the study and participated in its design and coordination, approved the final version of the manuscript. ND: conceived of the study and participated in its design and coordination, approved the final version of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

All authors read the manuscript and agree to the publication of the manuscript.

Ethics approval and consent to participate

Tumor tissue analyzed within this study had initially been collected for routine histo-pathological diagnostics. All diagnostic procedures had already been fully completed when samples were included in the study. All analyses were performed according to the standards set in the declaration of Helsinki 1975. Patient data were fully anonymized and the Ethics Committee of the Ludwig-Maximilians-University (Munich, Germany) approved the study (approval number 048-08). Researchers were blinded from patient data during experimental workup.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

¹Department of Obstetrics and Gynaecology - National Center for Tumor Diseases (NCT), Heidelberg University Hospital, Heidelberg, Germany. ²Department of Obstetrics and Gynecology, Ludwig-Maximilians-University of Munich, Munich, Germany. ³Department of Pathology, Ludwig-Maximilians-University of Munich, Munich, Germany. ⁴Department of Obstetrics and Gynecology, Technical University of Munich, Munich, Germany. ⁵Department of Internal Medicine, SLK-Kliniken Heilbronn GmbH, Heilbronn, Germany.

Received: 3 November 2016 Accepted: 17 March 2017

Published online: 20 April 2017

References

- Nadji M, Gomez-Fernandez C, Ganjei-Azar P, Morales AR. Immunohistochemistry of estrogen and progesterone receptors reconsidered: experience with 5,993 breast cancers. *Am J Clin Pathol*. 2005;123(1):21–7.
- Allred DC. Issues and updates: evaluating estrogen receptor-alpha, progesterone receptor, and HER2 in breast cancer. *Mod Pathol*. 2010;23 Suppl 2:S52–59.
- Tomao F, Papa A, Zaccarelli E, Rossi L, Caruso D, Minozzi M, Vici P, Frati L, Tomao S. Triple-negative breast cancer: new perspectives for targeted therapies. *OncoTargets Ther*. 2015;8:177–93.
- Perez EA, Romond EH, Suman VJ, Jeong JH, Sledge G, Geyer Jr CE, Martino S, Rastogi P, Gralow J, Swain SM, et al. Trastuzumab plus adjuvant chemotherapy for human epidermal growth factor receptor 2-positive breast cancer: planned joint analysis of overall survival from NSABP B-31 and NCCTG N9831. *J Clin Oncol*. 2014;32(33):3744–52.
- Yadav BS, Sharma SC, Chanana P, Jhamb S. Systemic treatment strategies for triple-negative breast cancer. *World J Clin Oncol*. 2014;5(2):125–33.
- Peshkin BN, Alabek ML, Isaacs C. *BRCA1/2* mutations and triple negative breast cancers. *Breast Dis*. 2010;32(1–2):25–33.
- Stevens KN, Vachon CM, Couch FJ. Genetic susceptibility to triple-negative breast cancer. *Cancer Res*. 2013;73(7):2025–30.
- Heublein S, Mayr D, Meindl A, Angele M, Gallwas J, Jeschke U, Ditsch N. Thyroid hormone receptors predict prognosis in *BRCA1* associated breast cancer in opposing ways. *PLoS One*. 2015;10(6):e0127072.
- Zhang K, Song L. Association between vitamin D receptor gene polymorphisms and breast cancer risk: a meta-analysis of 39 studies. *PLoS One*. 2014;9(4):e96125.
- Bonofiglio D, Cione E, Vizza D, Perri M, Pingitore A, Qi H, Catalano S, Rovito D, Genchi G, Ando S. Bid as a potential target of apoptotic effects exerted by low doses of PPAR γ and RXR ligands in breast cancer cells. *Cell Cycle*. 2011;10(14):2344–54.

11. Yasmin R, Kannan-Thulasiraman P, Kagechika H, Dawson MI, Noy N. Inhibition of mammary carcinoma cell growth by RXR is mediated by the receptor's oligomeric switch. *J Mol Biol.* 2010;397(5):1121–31.
12. Shankaranarayanan P, Rossin A, Khanwalkar H, Alvarez S, Alvarez R, Jacobson A, Nebbioso A, de Lera AR, Altucci L, Gronemeyer H. Growth factor-antagonized retinoid apoptosis involves permissive PPARgamma/RXR heterodimers to activate the intrinsic death pathway by NO. *Cancer Cell.* 2009;16(3):220–31.
13. Fischer C, Kuchenbacker K, Engel C, Zachariae S, Rhiem K, Meindl A, Rahner N, Dikow N, Plendl H, Debatin I, et al. Evaluating the performance of the breast cancer genetic risk models BOADICEA, IBIS, BRCAPRO and Claus for predicting BRCA1/2 mutation carrier probabilities: a study based on 7352 families from the German hereditary breast and ovarian cancer consortium. *J Med Genet.* 2013;50(6):360–7.
14. Ditsch N, Toth B, Himsl I, Lenhard M, Ochsenkuhn R, Friese K, Mayr D, Jeschke U. Thyroid hormone receptor (TR)alpha and TRbeta expression in breast cancer. *Histol Histopathol.* 2013;28(2):227–37.
15. Ditsch N, Mayr D, Lenhard M, Strauss C, Vodermaier A, Gallwas J, Stoeckl D, Graeser M, Weissenbacher T, Friese K, et al. Correlation of thyroid hormone, retinoid X, peroxisome proliferator-activated, vitamin D and oestrogen/progesterone receptors in breast carcinoma. *Oncol Lett.* 2012;4(4):665–71.
16. Ditsch N, Toth B, Mayr D, Lenhard M, Gallwas J, Weissenbacher T, Dannecker C, Friese K, Jeschke U. The association between vitamin D receptor expression and prolonged overall survival in breast cancer. *J Histochem Cytochem.* 2012;60(2):121–9.
17. Ditsch N, Vrekoussis T, Lenhard M, Ruhl I, Gallwas J, Weissenbacher T, Friese K, Mayr D, Makrigiannakis A, Jeschke U. Retinoid X receptor alpha (RXRalpha) and peroxisome proliferator-activated receptor gamma (PPARgamma) expression in breast cancer: an immunohistochemical study. *in vivo.* 2012;26(1):87–92.
18. Lenhard M, Lennerova T, Ditsch N, Kahlert S, Friese K, Mayr D, Jeschke U. Opposed roles of follicle-stimulating hormone and luteinizing hormone receptors in ovarian cancer survival. *Histopathology.* 2011;58(6):990–4.
19. Heublein S, Vrekoussis T, Mayr D, Friese K, Lenhard M, Jeschke U, Dian D. Her-2/neu expression is a negative prognosticator in ovarian cancer cases that do not express the follicle stimulating hormone receptor (FSHR). *J Ovarian Res.* 2013;6(1):6.
20. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumor marker prognostic studies. *J Clin Oncol.* 2005;23(36):9067–72.
21. 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. *J Natl Cancer Inst.* 2003;95(19):1482–5.
22. Livasy CA, Karaca G, Nanda R, Tretiakova MS, Olopade OI, Moore DT, Perou CM. Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. *Modern Pathol.* 2006;19(2):264–71.
23. Won JR, Gao D, Chow C, Cheng J, Lau SY, Ellis MJ, Perou CM, Bernard PS, Nielsen TO. A survey of immunohistochemical biomarkers for basal-like breast cancer against a gene expression profile gold standard. *Modern Pathol.* 2013;26(11):1438–50.
24. Satih S, Savinel H, Rabiau N, Fontana L, Bignon YJ, Bernard-Gallon DJ. Expression analyses of nuclear receptor genes in breast cancer cell lines exposed to soy phytoestrogens after BRCA2 knockdown by TaqMan Low-Density Array (TLDA). *J Mol Signal.* 2009;4:3.
25. Singh KK, Shukla PC, Yanagawa B, Quan A, Lovren F, Pan Y, Wagg CS, Teoh H, Lopaschuk GD, Verma S. Regulating cardiac energy metabolism and bioenergetics by targeting the DNA damage repair protein BRCA1. *J Thorac Cardiovasc Surg.* 2013;146(3):702–9.
26. Pickholtz I, Saadyan S, Keshet GI, Wang VS, Cohen R, Bouwman P, Jonkers J, Byers SW, Papa MZ, Yarden RI. Cooperation between BRCA1 and vitamin D is critical for histone acetylation of the p21waf1 promoter and growth inhibition of breast cancer cells and cancer stem-like cells. *Oncotarget.* 2014;5(23):11827–46.
27. Deng C, Ueda E, Chen KE, Bula C, Norman AW, Luben RA, Walker AM. Prolectin blocks nuclear translocation of VDR by regulating its interaction with BRCA1 in osteosarcoma cells. *Mol Endocrinol.* 2009;23(2):226–36.
28. Calvo V, Beato M. BRCA1 counteracts progesterone action by ubiquitination leading to progesterone receptor degradation and epigenetic silencing of target promoters. *Cancer Res.* 2011;71(9):3422–31.
29. Tsao WC, Wu HM, Chi KH, Chang YH, Lin WW. Proteasome inhibitors induce peroxisome proliferator-activated receptor transactivation through RXR accumulation and a protein kinase C-dependent pathway. *Exp Cell Res.* 2005;304(1):234–43.
30. Lattuada D, Viganò P, Mangioni S, Sassone J, Di Francesco S, Vignali M, Di Blasio AM. Accumulation of retinoid X receptor-alpha in uterine leiomyomas is associated with a delayed ligand-dependent proteasome-mediated degradation and an alteration of its transcriptional activity. *Mol Endocrinol.* 2007;21(3):602–12.
31. Lee WP, Jena S, Doherty D, Ventakesh J, Schimdt J, Furnick J, Widener T, Lemau J, Jurutka PW, Thompson PD. Sentrin/SUMO specific proteases as novel tissue-selective modulators of vitamin D receptor-mediated signaling. *PLoS One.* 2014;9(2):e89506.
32. Al-Azhri J, Zhang Y, Bshara W, Zirpoli G, McCann SE, Khoury T, Morrison CD, Edge SB, Ambrosone CB, Yao S. Tumor Expression of Vitamin D Receptor and Breast Cancer Histopathological Characteristics and Prognosis. *Clin Cancer Res.* 2017;23:97-103.
33. Berger U, McClelland RA, Wilson P, Greene GL, Haussler MR, Pike JW, Colston K, Easton D, Coombes RC. Immunocytochemical determination of estrogen receptor, progesterone receptor, and 1,25-dihydroxyvitamin D3 receptor in breast cancer and relationship to prognosis. *Cancer Res.* 1991;51(1):239–44.
34. Thakkar A, Wang B, Picon-Ruiz M, Buchwald P, Ince TA. Vitamin D and androgen receptor-targeted therapy for triple-negative breast cancer. *Breast Cancer Res Treat.* 2016;157(1):77–90.
35. Elstner E, Williamson EA, Zang C, Fritz J, Heber D, Fenner M, Possinger K, Koeffler HP. Novel therapeutic approach: ligands for PPARgamma and retinoid receptors induce apoptosis in bcl-2-positive human breast cancer cells. *Breast Cancer Res Treat.* 2002;74(2):155–65.
36. Wu K, Kim HT, Rodriguez JL, Hilsenbeck SG, Mohs SK, Xu XC, Lamph WW, Kuhn JG, Green JE, Brown PH. Suppression of mammary tumorigenesis in transgenic mice by the RXR-selective retinoid, LGD1069. *Cancer Epidemiol Biomarkers Prev.* 2002;11(5):467–74.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

