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Testing tumor type dependent relations between expression of ER and PgR with Ki-67 values in a single series of 1180 invasive ductal cancer patients

SVEN KURBEL[1]
BRANKO DMITROVIĆ[2]
KSENIJA MARJANOVIĆ[2]
BRANKA KRISTEK[3]

[1] Dept. of Internal Medicine, School of Medicine, Osijek, Croatia

[2] Dept. of Pathology and Forensic Medicine, School of Medicine, Osijek, Croatia

[3] Dept. of Radiology, School of Medicine, Osijek, Croatia

Correspondence:
Branka Kristek MD, PhD
School of Medicine
J Huttlera 4, 31000 Osijek, Croatia
E-mail: kristek.branka@kbo.hr

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Abstract

Background: *Model of cancer-associated epigenetic changes (Kurbel S. Tumour Biol. 2013;34:2011-7) proposes that dysfunctional estrogen receptors (ER), unable to adequately express progesterone receptors (PgR), beside in the ER^+PgR^- breast cancers might also be present in ER^+PgR^+ tumors showing weak PgR expression.*

Methods: *In 1180 patients with invasive ductal cancers, ER and PgR positivity were semiquantitatively classified in four groups: "0" means no positive cells; "1+" <10% positive cells; "2+" 11–30% positive cells; and "3+" 31–100% positive cells. Tumors were divided in breast cancer types.*

Results: *Among patients older than 54, Luminal A and B1 tumors were frequently ER^{3+} ($p<0.01$), while PgR^{3+} tumors were more common among Luminal A patients younger than 55 ($p=0.034$), suggesting that in older Luminal A or B1 patients, high ER and low PgR expression is common. Among Luminal B2 patients, ER and PgR expression did not depend much on age or on their Ki–67 values.*

The model predicted share of dysfunctional ERs was 7.32% (for Luminal A), 11.26% (B1), 12.62% (B2 & Ki–67<=20%) and 14.73% (B2 & Ki-67>20%). The predicted values matched well with the found shares of $ER^{3+}PgR^{1+}$ tumors within these three types ($p>0.10$).

Conclusions: *The results support heterogeneity among ER^+PgR^+ tumors. Future studies of ER^+PgR^+ phenotype variants are required since hypothetical dysfunctional ERs in some ER^+PgR^+ breast cancer patients might alter their endocrine treatment outcomes.*

1. INTRODUCTION

Epidemiologic data of breast cancer features were analyzed in a recent theoretic paper (1) proposing a model with two groups of breast cancer phenotype features.

The first group of features descends from the epigenetic phenotype of breast tissue cells that have become cancer cells. Possible examples of these breast tissue associated epigenetic features include HER2 absence or HER2 weak expression (1+ and 2+) in breast tumors.

The second proposed gropup consists of new features acquired during cancerogenesis due to the fact that these features are not present on

normal breast cells. A typical example among these cancer associated features is the HER2 overexpression (3+), since this level of HER2 expression is never found in normal breast ductal cells.

1.1 Functionality of estrogen and progesterone receptors

Several studies (cited in ref. *1*) have shown that ER on breast cancer cells can often be considered as less active, or dysfunctional. In short, possible ER/PgR combinations can be summarized like this:

– ER^+PgR^+ cancers: usually considered to have functional ERs and estrogen binding promotes the PgR expression.

– ER^+PgrR^- cancers: usually considered dysfunctional ER as they lack the ability to express PgRs.

– ER^-PgR^+ cancers: this rare phenotype can also be considered as a case of dysfunctional PgR expression despite the lack of detectable ER in tumor cells.

– ER^-PgR^- cancers: negative receptors suggest no estrogen dependency of tumor cells.

When considering estrogen receptor (ER) semiquantative expression, situation is more complex. Only a minority of normal breast cells has ERs *(2, 3),* suggesting that also only few of the progenitor cells are ER positive. In ref. 1 it was proposed that tissue specific ER expression has to be controlled by ligand binding, as it has been found in various normal estrogen target tissue *(4–11).* Two simple mechanisms are proposed to exist in cells with expressions of functional ER and PgR *(1, 4)*: the first is that estrogen binding to ER stimulates PgR expression in the same cell, and the second mechanism is that progesterone binding to PgR reduces ER and PgR expression in the same cell.

The proposed model *(1)* was based on the idea that if both mechanisms are active in tumor cells with the ER^+PgR^+ phenotype, this cancer can be considered to have "functional" ER and PgR receptors. Only these tumors are considered to have descended from ER^+ breast tissue progenitor cells, so their ER and PgR status is a tissue specific epigenetic feature.

If ER positive tumors have developed from the initially ER negative progenitor cells, their ER expression must have been acquired during cancerogenesis. Although it is possible that a fully functional ER and PgR expression can be acquired in this way, the model in ref. 1 is focused on the possibility that tumors arising from ER negative progenitor cells can often express dysfunctional ER receptors, unable to fully express PgR after estrogen binding. If the first of the proposed mechanisms is blocked, the resulting phenotype is ER^+PgR^- with "dysfunctional" ERs that do not promote the PgR expression after estrogen binding. This also means that in these ER^+ cells with scarce, or no PgR molecules, progesterone exposure cannot adequately reduce ER and PgR expressions.

The remaining phenotype is a rare ER^-PgR^+ that can be attributed to the tumor associated epigenetic PgR expression independent of estrogen binding to ER.

To apply this approach to reported data on steroid hormone receptors in breast cancer patients, reported data were in ref. 1 pooled from several studies. The results suggested that all ER^+PgR^-, ER^-PgR^+ and some of ER^+PgR^+ tumors possibly started from ER negative breast tissue progenitor cells.

1.2 The model predictions of ER functionality

Taken all together, the presence of functional ERs and functional PgRs can be considered as two separate events, each with its own probability. When tossing a fair coin, to calculate the probability of a certain outcome, number of times it appeared is divided by the number of tosses. With two coins, a certain combination of outcomes is expected to happen as a product of two probabilities.

So, if we are considering functional ER and functional PgR expression in breast cancers, the expected share of tumors with both receptors functional is in the model calculated by multiplying probability of ER^+ (p_{ER}) with probability of PgR^+ (p_{PgR}) in all breast tumors. Since among breast tumors with the "functional" ER^+PgR^+ phenotype, the probability of functional ER equals the functional PgR probability, the expected share of breast tumors with "functional" ER^+PgR^+ is the square of p_{PgR} *(1).*

The presented model uses this share as a conservative estimation of the expected share of tumors with the "functional" ER^+PgR^+ phenotype. Since the share of all ER^+PgR^+ tumors is larger, model suggests that a certain portion of tumors with this phenotype might be acquired during cancerogenesis and despite the presence of ERs and PgRs in tumor cells, one, or both receptors are possibly dysfunctional.

The cited model suggests that about 1/5 of all ER^+PgR^+ breast tumors in ref. 1 can be considered as an unexpected surplus of this phenotype, possibly linked to epigenetic ER expression in tumors developed from ER negative progenitor cells. This idea suggests that Luminal A and Luminal B are possibly more heterogenous tumor types than usually expected. This approach might explain variable response to endocrine therapy applied to all breast cancer patients with ER or PgR positivity.

Without a suitably detailed published data set, the only solution was to use a single institution experience in diagnosing more almost than 1200 IDC patients that has already been formed as a part of an ongoing research project (219–2192382–2426), financed by the Croatian Ministry of Science.

The aim of this study is to apply numeric methods described in the previous paper on real patient data and thus get more reliable answers on the possible mechanisms behind the breast cancer phenotypes.

2. PATIENTS, MATERIALS AND METHODS

2.1. Patients

In this study 1180 consecutive patients of ductal invasive breast carcinoma (any stage) were included. All patients were diagnosed and treated in Osijek Clinical Hospital from the time period January 2004 to December 2012. All of the specimens were excisional biopsies or mastectomy specimens. A tumor grade was determined using Bloom and Richardson sheme *(12, 13).*

2.2. Immunohistochemistry

Each immunostained slide was evaluated for the presence of ER and PgR expression, HER2 protein overexpression, and Ki–67 proliferation activity. Immunohistochemical staining was performed by standard avidin-biotin method (DAKO LSAB®2 System, HRP) using 4 μm sections from representative paraffin blocks. Nuclear staining with anti-ER, PgR, Ki-67 antibodies was sought and the percentage of positive cells per 500 tumor cells was calculated. Of note, all ER-positive and PgR-positive cases showed staining in at least 1% of the DCIS and/or invasive tumor cell nuclei, whereas all ER-negative and PgR-negative cases showed complete absence of tumor cell staining (but with staining of normal breast epithelial cell nuclei) *(14).*

ER and PgR cancer positivity was semiquantitative classified in 4 groups, according to the percentage of positive cells: "0" means no positive cells; "1+" up to 10% positive cells; "2+" 10% to 30% positive cells; and "3+" means 30% to 100% positive cells.

Tumor cells were considered positive for HER2 protein over-expression when greater than 10% of the cells showed strong membrane staining (equivalent to a score of 3+ in the DakoCytomation HercepTest). HER2 2+ result was only positive if confirmed by chromogene in situ hybridization for gene amplification. All immunostains were initially reviewed and scored by two of the study pathologists. Hormone receptors were reviewed and accepted as negative if 100% of cells lacked nuclear immunostaining for hormone receptor.

According to ICH analysis results', tumors were divided into following five groups: Luminal A (ER^+ and/or PgR^+, HER2-negative, Ki-67<=20%), Luminal B1 (ER^+ and/or PgR^+, HER2-negative, Ki-67>20%), Luminal B2 (ER^+ and/or PgR^+, HER2-positive, any Ki-67), HER2 (ER^-, PgR^-, HER2-positive), and triple-negative (ER^-, PgR^-, HER2-negative) *(15).*

2.3. Statistical analysis

Collected data were organized in 2x2 tables and differences from expected frequencies were checked by χ^2 tests.

Methods used for calculation of model predictions are based on equations described in the previous paper *(1),* also listed as formulas within the tables of this paper.

3. RESULTS

Since the tested model proposes that some of ER^+PgR^+ breast tumors got their ER during cancerogenesis started in steroid receptor negative progenitor cells, the first question was whether among our patients some HER2 or Ki67 subgroups differed in their ER or PgR expression.

3.1. Observed IHC phenotype features in breast cancer types

Table 1. shows collected data according to breast cancer types (listed in the top section of Table 1). Potentially the most diverse tumors are Luminal B2 that can have 6 phenotypic variants (3 steroid receptor phenotypes combine with low or high Ki-67 values). Both triple negative and pure HER2 overexpressed tumors are expected to have only two subgroups, depending on their Ki67 values. Luminal A and Luminal B1 tumors can come in three steroid hormone variants, since they are separated by their Ki67 value (Luminal A are up to 20% Ki67 positive, Luminal B1 have Ki67 values larger than 20%).

If we take this approach to our data (the middle section of Table 1) and calculate the expected frequencies (the bottom section of Table 1), the ER^+PgR^+ phenotype was more frequent than expected among our patients with low Ki67 values (457 pts. vs. expected 319.16), clearly suggesting that among our patients tumors with both receptors tend to have lower Ki67 values and lack HER2 overexpression. Among women with Luminal B1 tumors, the observed number of ER^+PgR^+ tumors was less than expected (177 vs. 261.13), due to small share of tumors with high Ki67 values among all ER positive tumors. In HER2 overexpressing tumors, number of ER^+PgR^+ tumors was less than expected, thus suggesting that among these patients HER2 overexpression might has been more important than the steroid receptors during cancerogenesis. A lack of ER^+PgR^+ tumors with low Ki67 value was observed among Luminal B2 patients (82 vs 120.29). Among ER^+PgR^- Luminal B2 tumors, the incidence of high Ki67 tumors exceeded the expected number (20 vs. 11.21).

Among pure HER2 overexpressed tumors, 98 of them showed high Ki-67 values, while only 34.37 were expected. On the other hand, among triple negative tumors high Ki67 values were found in 123 tumors, while it was expected to be only in 91.18, clearly suggesting that some HER2 and steroid receptor independent mechanism exists in these tumors and force them to increase their mitotic activity.

If we compare expected and observed frequencies, it is obvious that the statistical difference is mainly caused by receptor negative tumors (ER^-PgR^-) and tumors with both receptors (ER^+PgR^+). The other two combinations (ER^+PgR^- and ER^-PgR^+) did not differ much from the expected values when distributed by their HER2 and Ki-67 values.

If we take a close look on patients with ER^-PgR^- tumors, it is obvious that they had an increased share of tumors with high Ki-67 values (98 vs 34.37 for HER2 overexpressing tumor and 123 vs. 91.18 for HER2 negative tumors). These results also suggest that tumors lacking sex hormone receptors were more dependent on HER2 expression and other putative mechanisms behind their high Ki-67 values.

3.2. Distribution of semiquantatively estimated ER and PgR among breast tumor types

The next stage in analysing our patients data was to use semiquantitavely assesed ER and PgR presence in tumor tissue, instead the conventional positivity used in Table 1.

Table 2 shows distributions focused on age and Ki-67 as factors that affect distributions of ER or PgR:

- Reported frequencies of ER expression differed from the expected distribution according to age in older Luminal A and older Luminal B1 patients.
- Among 320 older Luminal A patients, 310 had ER^{3+} tumors ($p<0.0001$). It is interesting that PgR^{3+} expression in Luminal A patients was more common among younger patients, 102 out of 120 younger patients were PgR^{3+} ($p=0.034$). This finding suggest that both ER ligands and functional ERs were more common among younger Luminal A patients. In older patients ER functionality and ligand exposure might have been to low to stimulate PgR expression.
- In older Luminal B1 patients, 167 out of 175 patients were ER^{3+} ($p=0.0089$), while PgR expression in these patients was age unrelated ($p=0.1316$).
- In Luminal B2 patients, regardless of the Ki67 value, ER and PgR expressions were almost unrelated to age, suggesting that the ER functionality was less important in this tumor type, possibly due to HER2 overexpression.

TABLE 1

Distribution of invasive ductal breast cancer patients according to their immunohistochemical features.

Ki67 value (%)	HER2 overexpression	Breast tumor steroid receptor phenotypes				
		ER^+PgR^+	ER^+PgR^-	ER^-PgR^+	ER^-PgR^-	
Breast cancer types: phenotypic variants						
<=20%	Negative		Luminal A		Triple-negative	
	Positive		Luminal B2		HER2-over-exressed	
>20%	Negative		Luminal B1		Triple-negative	
	Positive		Luminal B2		HER2-over-exressed	
Number of breast cancer patients						Row Total
<=20%	Negative	457	33	6	31	527
	Positive	82	13	0	27	122
	Total	**539**	**46**	**6**	**58**	**649**
>20%	Negative	177	25	5	123	330
	Positive	83	20	0	98	201
	Total	**260**	**45**	**5**	**221**	**531**
Total		**799**	**91**	**11**	**279**	**1180**
Expected frequencies χ^2 =305.736, df=10, p<0.00001						Row Total
<=20%	Negative	319.16	36.35	4.39	111.45	471.35
	Positive	120.29	13.70	1.66	42.00	177.65
	Total	**439.45**	**50.05**	**6.05**	**153.45**	**649.00**
>20%	Negative	261.13	29.74	3.60	91.18	385.65
	Positive	98.42	11.21	1.36	34.37	145.35
	Total	**359.55**	**40.95**	**4.95**	**125.55**	**531.00**
Total		**799.00**	**91.00**	**11.00**	**279.00**	**1180.00**

TABLE 2

Age and Ki-67 dependent distributions of semiquantative ER and PgR expressions. Reduced ER expression was more common in younger Luminal A and Luminal B1 patients. In Luminal B1 patients high PgR expression was found in older patients, while in Luminal B2 patients ER and PgR expression did not depend much on age or on Ki-67.

Semiquantative steroid receptor expression	Ki-67 <=20%			Ki-67 >20%		
	Age <55 years	Age >54 years	Total	Age <55 years	Age >54 years	Total
Breast cancer type		**Luminal A**			**Luminal B1**	
ER $^{0-2+}$	16	10	26	12	8	20
ER^{3+}	104	310	414	76	167	243
Total	120	320	440	88	175	263
X^2(p)		16.36 (0.0001)			6.85 (0.0089)	
PgR $^{0-2+}$	18	78	96	24	64	88
PgR^{3+}	102	242	344	64	111	175
Total	120	320	440	88	175	263
X^2(p)		4.50 (0.034)			2.27 (0.1316)	
Breast cancer type			**Luminal B2**			
ER $^{0-2+}$	5	3	8	5	6	11
ER^{3+}	21	52	73	34	72	106
Total	26	55	81	39	78	117
X^2(p)		3.76 (0.0524)			0.80 (0.3703)	
PgR $^{0-2+}$	10	23	33	15	41	56
PgR^{3+}	16	32	48	24	37	61
Total	26	55	81	39	78	117
X^2(p)		0.08 (0.7741)			2.07 (0.1500)	

3.3. Model predictions of dysfunctional estrogen receptors

Table 3. is an attempt to estimate how much of the here presented patients might have acquired steroid receptors during cancerogenesis, beside the already recognized dysfunctional ER in the ER^+PgR^- phenotype.

Calculated results in Table 4. suggest that the greatest share of dysfunctional steroid receptors was expected in Luminal B2, particularly in the here proposed subtype with high Ki-67. For the comparison purposes only, the last rows shows numbers of patients with weak PgR phenotypes, and these data are not far from the predicted tumors with potentially dysfunctional ERs, suggesting that recognizing subtle difference in ER & PgR expression in breast tumors might be important.

4. DISCUSSION

Our understanding of the breast cancer occurrence largely depends on the idea that estrogen levels are associated with an increased risk for the development of breast cancer *(16,17).* Estrogen receptor (ER) and progesterone receptor (PR) status at the time of breast carcinoma surgery is used as a marker of both prognosis and hormone dependency to guide adjuvant therapy *(18).* Estrogen stimulates the proliferation of breast cancer cells and regulates the expression of other proteins, including the progesterone receptor *(19).*

It has been recognized that the presence of estrogen or progesterone receptors typically suggests slower-growing tumors, amenable to hormonal manipulation *(20).* Breast cancers classified by ER and PR status might represent diseases with different etiologies and clinical courses *(21-24).* However, most of the reported studies are inconclusive when considering this issue. It is reported that ER negative/PR positive breast carcinomas are biologically different from ER positive/PR positive tumors and have a poor clinical outcome *(21).* In 1556 breast cancer patients, risks regarding menstrual and reproductive (parity and lactation) characteristics, alcohol consumption, and family history were similar in patients with ER positive/PR positive and ER negative/PR negative tumors, while observed differences were related to age, race, and recreational exercise at 12–13 years of age *(23).* The conclusion was that these results only modestly support the hypothesis of two diseases with differing etiologies. In another study, levels of free and bound estradiol in archived blood samples were compared between postmenopausal breast

TABLE 3

An attempt to estimate how much of the here presented patients might have acquired steroid receptors during cancerogenesis (shown in shaded table fields), beside the already recognized dysfunctional ERs in the ER^+PgR^- phenotype. The expected numbers are smaller but proportional to the observed numbers of tumors with low PgR expression (shown in the bottom table section).

Steroid receptor positive breast cancer types			Symbols & formulas	Luminal A	Luminal B1	Proposed Ki-67/Luminal B2 subgroups	
						<=20%	>20%
Steroid hormone receptor phenotypes	tissue or cancerogenesis associated	ER^+PgR^+	Ph_1	**405**	**229**	**69**	**96**
	cancerogenesis associated	ER^+PgR^-	Ph_2	29	29	12	21
		ER^-PgR^+	Ph_3	6	5	0	0
	Total		C	440	263	81	117
Probability of tissue or cancerogenesis associated PgR expression *(D)*			Ph_1/C	0.9205	0.8707	0.8519	0.8205
Predicted breast cancers with **tissue associated ER^+PgR^+** phenotypes							
Predicted breast cancers with **tissue associated ER^+PgR^+** phenotypes	Predicted share (%)		D^2	84.72%	75.82%	72.57%	67.32%
	Predicted number of patients with functional ER		D^2*C	**372.8**	**199.4**	**58.8**	**78.8**
Observed frequencies of ER^+PgR^+ phenotypes with full PgR expression							
Number of patients with:	ER^{3+} and PgR^{3+} phenotype			325	168	45	58
	ER^{2+} and PgR^{3+} phenotype			8	4	3	1
	ER^{1+} and PgR^{3+} phenotype			5	3	0	2
Total				**338**	**175**	**48**	**61**
Predicted breast cancers with **cancerogenesis associated ER^+PgR^+** phenotypes							
Predicted breast cancers with **cancerogenesis associated ER^+PgR^+** phenotypes	Predicted share (%)		$E=D-D^2$	7.32%	11.26%	12.62%	14.73%
	Predicted number of patients with potentially dysfunctional ER *($dysf._{pred}$)*		$E*C$	**32.2**	**29.6**	**10.2**	**17.2**
Observed frequencies of ER^+PgR^+ phenotypes with reduced PgR expression							
Number of patients with:	ER^{3+} and PgR^{1+} phenotype			40	26	11	24
	ER^{3+} and PgR^{2+} phenotype			23	24	6	6
	ER^{2+} and PgR^{1+} phenotype			2	2	1	2
	ER^{2+} and PgR^{2+} phenotype			0	0	2	1
	ER^{1+} and PgR^{1+} phenotype			2	0	1	1
	ER^{1+} and PgR^{2+} phenotype			0	2	0	1
Total number of tumors with low PgR expression				**67**	**54**	**21**	**35**

cancer patients and matched controls *(22)*. The results showed that the association of endogenous estrogens with breast cancer risk is independent of the tumor ER status. These results were also interpreted as more compatible with the hypothesis of a progression from ER positive to ER negative tumors than with the hypothesis that ER status identifies two distinct types of breast cancer. Results that link tamoxifen and raloxifen as selective estrogen receptor modulators (SERM) with the reduced occurrence of only ER positive tumors strongly support the etiological distinction between ER positive and ER negative breast cancers. Tamoxifen prevention trials showed no effect for ER negative breast cancers, but ER positive cancers were decreased by 48% *(25)*. In 8981 breast cancer patients older than 50, tamoxifen decreases only the risk of ER positive contralateral breast tumors (26). Raloxifene was shown to reduce the risk of both in situ and invasive breast cancer by 65%, with the most significant risk reduction in women who developed ER positive cancers *(27)*.

It was reported that despite the menopausal status, the expression of ER and PR in normal breast tissue is highly variable, with many apparently negative cells *(28)*. In premenopausal normal breast tissue, 6% of cells are ER positive and 29% PR positive *(29)*. Beside that, expression of ER declines in the normal breast tissue as the men-

strual cycle progresses *(30)* and tamoxifen therapy increases the mean percentage ER positivity in normal ductal tissue *(28).* During breast development, ER and PR are almost absent in fetal and infant breasts, while their expression is high in the epithelial cells of the pubertal breast (31). Levels of mRNA expression of the ER gene depend on the hormonal status, with relatively higher levels in breasts of perimenarchal girls, and women in the luteal phase of the menstrual cycle, and in those with fibrocystic changes *(32).*

4.1. The role of dysfunctional ER

This surplus of ER^+PgR^+ tumors among tumors with low HER2 expression and low mitotic activity suggests two things:

a) some of these tumors might have originated from ER negative progenitor cells and later aquired their ER;

b) phenotypic change due to ER expression might have been particularly important in cancerogenesis of these tumors.

From several studies *(6–12),* it is well established that PgR expression in cells with ER depends on estrogen exposure during previous days, suggesting that ligand binding to ER is a physiologic prerequisite of PgR expression and the accepted concept is that ER^+PgR^- breast tumor phenotype is caused by dysfunctional ER action upon ligand binding.

When considering progesterone binding to PgR, several reports show that progesterone exposure diminishes both ER and PgR expression in target tissues. Lundgren *et al. (8)* have reported that high dose oral gestagen diminishes PgRs and reduces ERs and androgen receptors in the breast cancer tissue. Vereide *et al. (12)* have found in uterine mucosa that gestagen reduces glandular and stromal PgRs and ERs. Likewise, it is mentioned in the textbook by Boron and Boulpaep *(20)* that "Progestins are also antiestrogens. As a result, progestins acting locally may downregulate estrogen receptors and reduce the effectiveness of estradiol."

These two interactions between ligand binding and steroid receptor expressions in estrogen dependent tissues, can make a solid regulatory frame during the ovulatory cycle *(5).* Preovulatory estrogen exposure initiates tissue activity and increases PgR presence. During the luteal phase, progesterone binding to PgR slowly diminishes both ER and PgR expression in target tissues and diminishes all estrogen-induced actions, if no pregnancy has occurred.

Here tested model proposes that ligand binding to dysfunctional ERs can also lead to weak PgR expression, allowing that some of ER^+PgR^+ tumors might also have dysfunctional ERs. As an explanation of dyscrepancy between to probably very few ER^+ progenitor cells in breast ducts and many ER^+ ductal cancers, a proposition is made that most of the tumors with dysfunctional ERs come from ER^- progenitor cells that acquired ER expression during cancerogenesis. In this case, weak or absent PgR expression upon the ER ligand binding is considered much more probable than in breast tumors developed from ER^+ progenitor cells.

4.2. Possible differences in tumor biology of tumors with dysfunctional ERs

In Luminal B1 tumors, an increased share of $ER^{3+}PgR^{3+}$ phenotype was found in all patients and particularly in patients older than 54 years. Only in Luminal B2 with Ki-67 values >20%, an increased share of $ER^{3+}PgR^{3+}$ tumors was found in older patients (). A possible explanation is that functional ER are upregulated in the menopausal setting, estrogen binding helps express PgR, while the lack of progesterone exposure allows high ER and PgR levels. Among 397 Luminal A patients, reduced ER expression was more common in younger patients ($p<0.0001$), possibly reflecting premenopausal hormone exposures. A similar situation was with Luminal B1 patients ($p=0.0382$). In Luminal B1 patients high PgR expression was found in older patients ($p=0.0337$), possibly due to lack of progesterone exposure.

The model predicts that at least 39 of these 301 ER^+PgR^+ patients might have potentialy dysfunctional ERs. If estrogen binding to ER in these tumors only partially express PgR, the reduced PgR availability diminishes progesterone suppression of ER and PgR expression, making tumor cells more susceptible to estrogen stimulation.

The greatest share of model predicted dysfunctional ER^+PgR^+ tumor phenotypes was expected in Luminal B2, particularly in these tumors with Ki-67 >20% and predicted numbers correlate with the number of patients with $ER^{3+}PgR^{1+}$ tumors.

It seems that estrogen binding to ER in some Luminal A, B1, or B2 tumors only partially expresses PgR, resulting in a dysfunctional variant of the ER^+PgR^+ phenotype. If this reduces progesterone suppression of ER and PgR expressions, these tumor cells can become more susceptible to estrogen stimulation.

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