ISSN 1669-9106

SPECIAL ARTICLE - REVIEW

MEDICINA (Buenos Aires) 2019; 79 - N° 6/1 -
NÚMERO ESPECIAL 80 ANIVERSARIO: 540-545

HORMONE RECEPTORS IN BREAST CANCER: MORE THAN ESTROGEN RECEPTORS

CAROLINE A. LAMB[1], SILVIA I. VANZULLI[2], CLAUDIA LANARI[1]

[1]*Laboratorio de Carcinogénesis Hormonal, Instituto de Biología y Medicina Experimental (IBYME-CONICET),*
[2]*Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina, Buenos Aires, Argentina*

Abstract Seventy per cent of breast cancers are luminal carcinomas that express estrogen receptor alpha (ER). For several decades, its expression has been used as a therapeutic target in patients with breast cancer. These therapies are aimed at blocking ER or inhibiting ligand synthesis. The expression of progesterone receptors (PR) is evaluated as a prognostic factor together with ER. It has been shown that there are two predominant PR isoforms with different molecular weight, isoform A and isoform B, which are not distinguished by immunohistochemical techniques. The available evidence indicates that the PR isoform ratio may have both a prognostic and predictive value of the response to antiprogestin treatment. In luminal mammary carcinomas, androgen receptors (AR) are expressed in a high percentage and the AR/ER or AR/PR ratio could be a prognostic factor. In ER negative (-) tumors, AR expression is an indicator of poor prognosis and it is proposed that they may be susceptible to antiandrogen treatment. Finally, the expression of glucocorticoid receptors (GR) would be an indicator of good or bad prognosis in luminal or ER- tumors, respectively. In ER- tumors, metastases express higher levels of nuclear GR than primary tumors and therapies that block GR could improve the efficacy of chemotherapy. Given the crosstalk of pathways triggered by different hormone receptors, it is possible that in the future, a therapeutic scheme can be administered that contemplates the expression of ER, PR isoforms, AR and GR.

Key words: breast cancer, estrogen receptors, progesterone receptor isoforms, androgen receptors, glucocorticoid receptors

Resumen ***Receptores hormonales en cáncer de mama: receptores de estrógenos y algo más.*** El 70 por ciento de los carcinomas mamarios son luminales y expresan receptores de estrógenos alfa (RE). Desde hace varias décadas, su expresión se utiliza como blanco terapéutico en pacientes con cáncer de mama. Estas terapias están dirigidas a bloquear el RE o a inhibir la síntesis del ligando. La expresión de receptores de progesterona (RP) se evalúa como factor pronóstico junto con los RE. Se ha comprobado que existen dos isoformas predominantes de RP de distinto peso molecular, isoforma A e isoforma B, que no se distinguen por técnicas de inmunohistoquímica. Las evidencias indican que la proporción de isoformas de RP podría tener tanto un valor pronóstico como predictivo de la respuesta a un tratamiento con antiprogestágenos. En tumores mamarios luminales, los receptores de andrógenos (RA) se expresan en un alto porcentaje y la proporción de RA/RE o RA/RP podría ser un factor pronóstico. En tumores RE-, la expresión de RA es indicador de mal pronóstico y se propone que serían susceptibles a un tratamiento con antiandrógenos. Por último, la expresión de receptores de glucocorticoides (RG) sería un indicador de buen o mal pronóstico en tumores luminales o RE-, respectivamente. En tumores RE-, las metástasis expresan mayores niveles de RG nuclear que los tumores primarios y las terapias que bloquean los RG podrían mejorar la eficacia de la quimioterapia. Dado los entrecruzamientos de vías gatilladas por distintos receptores hormonales es posible que en un futuro se pueda administrar un esquema terapéutico que contemple la expresión de RE, isoformas de RP, RA y RG.

Palabras clave: cáncer de mama, receptores de estrógenos, isoformas de receptor de progesterona, receptor de andrógenos, receptor de glucocorticoides

Statistics indicate that one out of eight women will develop breast cancer throughout their lives. From the clinical point of view, it is essential to know the age, personal and family history of breast and ovarian cancer, as well as the tumor size, the presence of axillary lymph nodes and the time elapsed from the first symptom to the consultation. Once the biopsy or surgery is performed, a histopathological examination is carried out to classify the tumor type and establish the histological grade. These characteristics, together with the biomarkers measured by immunohistochemistry (IHC), provide the diagnosis and constitute prognostic and predictive values of therapeutic response. Once the tumor is categorized and the patient is staged, it is possible to evaluate the risk of relapse in order to make the appropriate therapeutic decisions in each case.

The biomarkers that are currently used include the determination of estrogen receptor alpha (ER) and proges-

Received: 2-VII-2019 Accepted: 23-VII-2019

Postal address: Claudia Lanari, Laboratorio de Carcinogénesis Hormonal (LCH), Instituto de Biología y Medicina Experimental (IBYME), Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina
e-mail: lanari.claudia@gmail.com

terone receptors (PR), overexpression of the epidermal growth factor receptor type 2 (HER2 or c-erb-B2) and the expression level of the cell proliferation marker, Ki-67. These biomarkers are evaluated by IHC and, when the result of HER2 is doubtful, gene amplification is verified by *in situ* hybridization techniques. In the case of hormone receptor positive tumors with moderate Ki-67 index, where it is difficult to determine the benefit of chemotherapy, molecular platforms are used. These platforms report high or low risk scores that collaborate with oncologists in making decisions. Currently, the most used and accepted genomic test in Argentina is the Oncotype DX. This assay measures the expression of 21 genes by gene amplification (RT-PCR). The expression of 16 oncogenes and 5 reference genes is evaluated and a score assigned to the result that informs about the risk of disease recurrence. If the score is less than 26, the risk of recurrence would be low and, therefore, the administration of chemotherapy is not advised. On the other hand, if the score obtained is greater than or equal to 26, chemotherapy is recommended. For premenopausal patients, there is a range of uncertainty for samples with a score between 18 and 30 (breastcancer.org).

Most (70-75%) breast carcinomas express ER and PR and, according to the molecular classification, these tumors correspond to the luminal type. In turn, these tumors are subclassified in luminal A (Lum A) or B (Lum B) according to whether they have a low or high rate of proliferation, respectively[1]. The latter, in addition to expressing ER, usually express lower PR levels compared with Lum A tumors and may also express HER2. ER+/PR- breast carcinomas are also included in this group (Table 1). From the clinical point of view, they arouse special interest, since they have a worse prognosis than ER+/PR+ tumors. It is postulated that these tumors have exacerbated growth factor signaling pathways and specific mutations associated with this phenotype have been described[2]. Luminal tumors have a better prognosis than the rest of breast carcinomas and they can respond to endocrine therapies aimed at blocking ER (tamoxifen or fulvestrant) or, at inhibiting the endogenous production of their natural ligands (aromatase inhibitors). The group of tumors that do not express ER, is formed by a subgroup that overexpresses HER2, and therefore is susceptible to a therapy with antibodies directed to block this receptor, or by tumors called triple negative (TN) since they do not

TABLE 1.– *Frequency of mammary carcinomas according to the expression of hormonal receptors and HER2 and their corresponding molecular classificatio*n

ER	%	Tumor subtype based on PR and HER2 expression	%*	%*	%**	Molecular classification**
ER+	73.5	ER+PR+	66.6			
		ER+PR+HER2-		89	59.3	Lum A (Ki-67< 14) or Lum B (Ki-67 ≥ 14)
		ER+PR+HER2+		11	7.3	Lum B
		ER+PR-	6.9			
		ER+PR-HER2-		65.5	4.5	Lum B
		ER+PR-HER2+		34.5	2.4	Lum B
ER-	26.5	ER-PR+	1.3			
		ER-PR+HER2-		65.5	0.9	Similar to TN
		ER-PR+HER2+		34.5	0.4	Similar to TN o Her2 +
		ER-PR-	25.2			
		ER-PR-HER2-		61.2	15.4	TN: basal-like immune associated (BLIA), basal-like immune suppressed (BLIS), luminal androgen receptor (LAR) and, mesenquimal-like (MES)
		ER-PR-HER2+		38.8	9.8	HER2

**Percentages obtained from Bae et al,* BMC Cancer *2015; 15: 1138*

***Molecular classification of Perou, Sorlie et al,* Nature *2000; 406: 747-52; Burstein et al,* Clin Cancer Res *2015; 21: 1688-98.*

express ER, PR or HER2. According to the molecular classification, TN tumors include basal tumors. These tumors have the worst prognosis[1] (Table 1) and do not yet have a standardized targeted therapy. On the other hand, there is a controversy regarding ER-/PR+ tumors. This group accounts for less than 2% of breast cancers and the controversy relies in the fact that some researchers consider them as an independent group with their own characteristics, while others consider that diagnostic errors, either by PR over-valuation or ER sub-valuation, led to their classification, since when several cases were reviewed, many of them changed their category[3]. Likewise, the study of this group determined that the cell proliferation rate, the percentage of P53 mutations and overall patient survival resemble TN tumors more than luminal tumors[4].

Hormone receptors

ER, PR, androgen (AR), mineralocorticoid, glucocorticoid (GR), retinoic acid, and other less-known receptors belong to the steroid receptor superfamily. They share a similar structure that include hormone binding, nuclear translocation, DNA binding and transactivation domains. These receptors are activated after high affinity hormone binding and, once active, they behave as transcription factors by binding to the promoters of target genes at specific binding sites. Alternatively, they can be activated in the absence of ligand since they can be phosphorylated by kinases such as MAPK and AKT, which are usually overactivated in neoplastic processes. The activated receptors can in turn act as coactivators, potentiating the effects of other transcription factors. In recent years, it has been shown that the different receptors can interact with each other in the promoters of the target genes, showing a higher level of complexity in the regulation of biological responses[5-8]. Unraveling the mechanism of action of steroid hormones has been of great help to understand the mechanisms related to endocrine resistance.

Considering that endocrine therapies are much less invasive than nonspecific therapies such as chemotherapy, it is extremely important to determine the mechanisms underlying hormone-regulated tumor growth and, whether blocking other hormone receptors, different from ER, could be an additional therapeutic alternative to improve the effects of current endocrine therapies.

Estrogen receptors

Elwood Jensen (1920-2012) can be considered as the father of hormone receptors, especially ER. Then, J. Gorski and G. Greene made great contributions demonstrating that these receptors had a predominant nuclear location (reviewed in[9]). Currently, the presence of ER in breast carcinomas is determined by IHC. These are considered ER+ if the staining is located in the cell nucleus while cytoplasmic staining is ignored. However, more than thirty years ago, receptors were evaluated by binding techniques, that consisted in measuring the ability of the receptors to bind radioactive hormone with high affinity in a cell extract. At that time, this method did not distinguish if more than one type of receptor bound to the hormone or, if different cell populations bound estradiol with the same affinity. Subsequently, in the nineties, Jan-Åke Gustaffson discovered a different estrogen receptor that binds estrogens with high affinity. This novel receptor was named ER beta to differentiate it from the already known ER alpha[10]. While these two isoforms are encoded by genes located on different chromosomes, they share a high sequence homology. The role of ER beta in breast cancer is not clear and it is not evaluated in the clinic.

The antibodies that are used in diagnostic pathology specifically assess expression of ER alpha. However, its detection by IHC cannot distinguish whether the receptor is functional, mutated, or if it is a lower molecular weight isoform that maintains the epitope recognized by the antibody.

As we mentioned in previous paragraphs, ER determination is important not only because it is an independent prognostic marker, but also because it is a predictive marker of response to treatment. Patients with breast tumors with ER expression levels greater than or equal to 1% can be treated with endocrine therapy. However, in a patient with a tumor with 99% of ER- cells, a worse response to treatment is expected compared to those tumors in which all their cells express ER.

For a long time, Fuqua et al. have postulated that one of the mechanisms that explain endocrine therapy resistance is the presence of mutated ERs[11]. At the time, no correlation was found between the presence of these mutations and the response to treatment, and this theory was not sustained. Interestingly, in the last few years this concept has resurfaced since metastases in many patients express mutated ER that were not present in the original tumor[12].

Progesterone receptors

Why are PRs evaluated if, until now, there is no treatment aimed at blocking them? What is the benefit of their quantification? It has been shown that, in ER+ mammary carcinomas, PR quantification has prognostic value. As previously mentioned, the ER+/PR- subgroup is of worse prognosis, either because it is a different entity or because it implies that ERs are not functional since one of the physiological effects of ER is to induce PR synthesis. In the same way as for ERs, they were formerly evaluated by ligand binding techniques and they are currently measured by IHC. The PR gene codes for at least two major proteins, isoform B (PRB) of higher molecular weight and isoform A (PRA) which is a truncated protein that lacks the first

161 amino acids. Since PRA is included within PRB, it is very difficult to have antibodies that exclusively recognize PRA. There are few studies in which the expression of both isoforms has been quantified (reviewed in [13]). The consensus is that in the normal mammary gland the same level of either PR isoform is expressed, whereas this ratio is altered in tumors, where PRA often predominates over PRB. Some authors propose that tumors with a higher proportion of PRA than PRB would have a lower response to tamoxifen and, therefore, worse prognosis (reviewed in [13]). On the contrary, in our laboratory we have shown that tumors with a higher proportion of PRA than PRB share characteristics with Lum A tumors and, consequently, they would have a better prognosis than those with the opposite proportion, which are similar to Lum B. Moreover, the latter are tumors with a higher cell proliferation rate and lower total PR expression[14]. Likewise, the results of our laboratory show that tumors with higher levels of PRA than PRB respond *ex vivo* to treatment with antiprogestins such as mifepristone[14]. These studies suggest that PR could also be used as a therapeutic target to be used in conjunction with current endocrine therapy in patients selected according to their PR isoform profile.

On the other hand, it should be noted that progestins have been used in high concentrations in the eighties and other authors have proposed their use in combination with conventional endocrine therapy. Currently, there are several ongoing studies in the clinic testing the progestin megestrol acetate in patients with advanced breast cancer. The challenge in this field is to distinguish which patients would benefit from either treatment.

Androgen receptors

ARs are not routinely measured in breast cancer patients. Two isoforms and several alternative splicing variants, encoded by the same gene, have been described. The most commonly observed variant is the one with the highest molecular weight, which is expressed in approximately 60-70% of mammary carcinomas[15]. Considering that an interrelation between ER and AR has been demonstrated[16], determining AR expression was attractive not only to explore AR as an additional therapeutic target, but also as a prognostic biomarker in breast cancer. In fact, recent studies have determined that the ratio AR/ER > 2 is associated with a worse response to treatment with tamoxifen and a worse disease-free survival, so that the measurement of this relationship could have prognostic value.

The current consensus is that it is a marker of good prognosis in luminal tumors and a worse prognosis marker in TN breast cancer[17] (Table 2). This subgroup of TN tumors expressing AR has been cataloged as a LAR subtype (Luminal Androgen Receptor), and it has been observed that these patients have a lower disease-free survival in response to conventional treatments. However, the expression of AR in these tumors could make them susceptible to antiandrogen treatment.

In 1988, flutamide was the first AR antagonist tested in patients with breast cancer, but it did not prosper, probably due to its side effects and lack of clear response. Years later, interest was rekindled with AR determination in breast cancer and the development of new drugs for prostate cancer treatment. In trials with bicalutamide, better responses were obtained in patients selected for having more than 10% AR and, currently, there are several studies that use third generation AR antagonists, such as enzalutamide, and other AR modulators, such as enobosarm, in combination with aromatase inhibitors.

Glucocorticoid receptors

The GRs are encoded by the NR3C1 gene. This gene codes for several isoforms with different cell localization and distinct biological functions. Like ARs, GRs are not currently evaluated in breast carcinomas; however, numerous studies suggest that using these receptors as a therapeutic target may have certain benefits.

In a retrospective meta-analysis study with more than 1000 ER+ patients in early stages, it was observed that high GR mRNA levels were associated with a better prognosis compared to patients with low or no GR expression. However, the opposite was observed when evaluating 300 ER- patients in whom GR expression was associated with a worse prognosis[18] (Table 2). These results are very similar to those reported for AR. Likewise, it was demonstrated that there is a cooperation between ER and GR so that when both receptors are activated there is a greater positioning of these receptors in genes related to cell differentiation[19]. Given that GR expression was associated with survival and chemoresistance genes in TN tumors, one study suggests treatment with GR antagonists together with chemotherapy in order to improve the therapeutic effect[20].

In conclusion, given the interplay between different steroid receptor signaling pathways, it is extremely important to evaluate their role both in luminal and TN tumors since, in the absence of ER, the AR and GR could have opposite functions. We can envisage that in the future, the determination of PR isoforms, as well as the determination of AR and GR, will allow us to classify tumors as quadruple or quintuple positive or negative, adding other prognostic and/or predictive factors for treatments with endocrine agonists/antagonists.

TABLE 2.– *AR and GR in mammary carcinomas with or without ER and PR expression*

Hormone receptors		Experimental data	Clinical data
ER+PR+			
	AR+[1]	*In vitro* • Androgens: ↓ cell proliferation at low concentrations and ↑ at high concentrations • AR antagonists: ↓ cell proliferation *In vivo* • Androgens and AR antagonists: ↓ tumor growth	• > disease free survival and overall survival, especially in Lum A • AR/ER ≥ 2.0 higher risk to failure to tamoxifen therapy. A similar trend is seen with AR/PR
	GR+[2]	*In vitro* • Dexa* (1 μM): ↓ cell proliferation and cell migration in MCF-7 cells *In vivo* • Dexa (0.1 mg/kg; ip, 2x/week): ↓ tumor growth (MCF-7)	• > GR: ↑ overall survival
ER-PR-			
	AR+[1]	*In vitro* • Androgens: ↑ cell proliferation. ↑ HER2 mRNA. HER2 blockage ↓ androgen response • AR antagonists: ↓ cell proliferation *In vivo* • Androgens: ↑ tumor growth • AR antagonists: ↓ tumor growth	• HER2+: unclear role of AR • TN: Some studies report better and others worse overall survival
	GR+[2]	*In vitro* • Dexa: ↓ cell proliferation and cell migration in MDA-MB-231 cells *In vivo* • Dexa: ↓ tumor growth • Metastasis: ↑ GR nuclear localization	• Metastasis: > GR nuclear localization • GR antagonists: improve the effect of chemotherapy • > GR: ↓ overall survival

**Dexa: dexamethasone, glucocorticoid agonist*
[1]*Venema et al, Pharmacol Therap 2019, in press;* [2]*Kumar et al, Carcinogenesis 2019; 40: 335-48*

Acknowledgments: This work was funded by ANPCYT, PICT 2015-1022; PICT 2017-2073, INC 2017, *Fundación para el Progreso de la Medicina en Córdoba*, and Fundación Gador.

Conflicts of interest: None to declare

References

1. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature* 2000; 406: 747-52.
2. Lopez G, Costanza J, Colleoni M, et al. Molecular insights into the classification of luminal breast cancers: The genomic heterogeneity of progesterone-negative tumors. *Int J Mol Sci* 2019; 20: 510.
3. Schroth W, Winter S, Buttner F, et al. Clinical outcome and global gene expression data support the existence of the estrogen receptor-negative/progesterone receptor-positive invasive breast cancer phenotype. *Breast Cancer Res Treat* 2016; 155: 85-97.
4. Bae SY, Kim S, Lee JH, et al. Poor prognosis of single hormone receptor- positive breast cancer: similar outcome as triple-negative breast cancer. *BMC Cancer* 2015; 15: 138.

5. Giulianelli S, Vaque JP, Soldati R, et al. Estrogen receptor alpha mediates progestin-induced mammary tumor growth by interacting with progesterone receptors at the cyclin D1/MYC promoters. *Cancer Res* 2012; 72: 2416-27.
6. Mohammed H, Russell IA, Stark R, et al. Progesterone receptor modulates ERalpha action in breast cancer. *Nature* 2015; 523: 313-7.
7. Sikora MJ. Family Matters: Collaboration and conflict among the steroid receptors raises a need for group therapy. *Endocrinology* 2016; 157:4553-60.
8. Giulianelli S, Vaque JP, Wargon V, et al. [The role of estrogen receptor alpha in breast cancer cell proliferation mediated by progestins]. *Medicina (B Aires)* 2012; 72: 315-20.
9. O'Malley BW, Khan S. Elwood V. Jensen (1920-2012): father of the nuclear receptors. *PNAS* 2013; 110: 3707-08.
10. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. *PNAS* 1996; 93: 5925-30.
11. McGuire WL, Chamness GC, Fuqua SA. Estrogen receptor variants in clinical breast cancer. *Mol Endocrinol* 1991; 5: 1571- 77.
12. Toy W, Shen Y, Won H, et al. ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. *Nat Genet* 2013; 45: 1439-45.
13. Lamb CA, Fabris VT, Jacobsen B, Molinolo AA, Lanari C. Biological and clinical impact of imbalanced progesterone receptor isoform ratios in breast cancer. *Endocr Relat Cancer* 2018; ERC-18-0179.
14. Rojas PA, May M, Sequeira GR, et al. Progesterone receptor isoform ratio: A breast cancer prognostic and predictive factor for antiprogestin responsiveness. *J Natl Cancer Inst* 2017; 109: djw317.
15. Gucalp A, Traina TA. Targeting the androgen receptor in triple-negative breast cancer. *Curr Probl Cancer* 2016; 40: 141-150.
16. Peters AA, Buchanan G, Ricciardelli C, et al. Androgen receptor inhibits estrogen receptor-alpha activity and is prognostic in breast cancer. *Cancer Res* 2009; 69: 6131-6140.
17. Vera-Badillo FE, Templeton AJ, de Gouveia P, et al. Androgen receptor expression and outcomes in early breast cancer: a systematic review and meta-analysis. *J Natl Cancer Inst* 2014; 106: djt319.
18. Pan D, Kocherginsky M, Conzen SD. Activation of the glucocorticoid receptor is associated with poor prognosis in estrogen receptor-negative breast cancer. *Cancer Res* 2011; 71: 6360-6370.
19. West DC, Pan D, Tonsing-Carter EY, et al. GR and ER coactivation alters the expression of differentiation genes and associates with improved ER+ breast cancer outcome. *Mol Cancer Res* 2016; 14: 707-19.
20. Skor MN, Wonder EL, Kocherginsky M, et al. Glucocorticoid receptor antagonism as a novel therapy for triple-negative breast cancer. *Clin Cancer Res* 2013; 19: 6163-72.

- - - -

Para el investigador básico, relatar sus experimentos no resulta tarea sencilla. Suele sumergirse en ellos, en la "torre de marfil", aislado de la realidad cotidiana acompañado por la ahora infaltable computadora. Por lo general, el lego piensa que se trata de un varón, un excéntrico distraído, nunca una mujer. Pero hubo mujeres científicas, de a cuenta gota en los tiempos lejanos, hasta que poco a poco penetraron en "el mundo del investigador" –como me gusta denominarlo– y hoy ya se destacan cada vez más.

Christiane Dosne Pasqualini

En: Quince mujeres recibieron el Premio Nobel en ciencia (Editorial).
Medicina (B Aires) 2013; 73: 277-9