BRCAness and breast cancer

BRCAness y cáncer de mama

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BREAST CANCER CONSTITUTES THE NEOPLASM OF HIGHEST INCIDENCE, PREVALENCE AND MORTALITY AMONG WOMEN IN WESTERN COUNTRIES. AROUND 25-30% OF THESE TUMORS ARE ESTROGEN RECEPTOR (ER), PROGESTERONE RECEPTOR (PR) AND HER2 NEGATIVE. THESE TUMORS ARE CALLED TRIPLE NEGATIVE BREAST CANCER (TNBC). TNBCS FREQUENTLY APPEAR IN YOUNG PATIENTS. FAMILIAL AGGREGATION IS COMMON. THEY SHOW A HIGH HISTOLOGICAL GRADE AND LARGER LYMPH NODE LOCO-REGIONAL AFFECTATION WHEN DIAGNOSED. IN ADDITION, THEY RECUR AND PROGRESS PREMATURELY AFTER THE STANDARD COMBINED TREATMENT.

A PART OF THESE TNBCS -UP TO 50% ACCORDING TO SOME AUTHORS- SHOW SPECIFIC PHENOTYPICAL AND MOLECULAR FEATURES THAT ALLOW THEM TO BE CONSIDERED BRCANESS POSITIVE. BRCANESS ENCOMPASSES A COMBINATION OF CHANGES, OF VARIED NATURE. THESE CHANGES POINT TOWARDS A DEFICIT IN HOMOLOGY-DIRECTED REPAIR (HDR). THESE TNBC TYPES ARE GENERATED AND ENSURE THEIR SURVIVAL THROUGH THE ACCUMULATION OF UNREPAIRED DAMAGE THAT INCREASES GENOME INSTABILITY.

RECENTLY IT HAS BEEN DEMONSTRATED THAT THESE TUMORS COULD BE SUSCEPTIBLE TO SPECIFIC TREATMENTS (ALKYLATING AGENTS, PARP INHIBITORS) WITH BETTER RESPONSE AND SURVIVAL RATES. THIS LEADS TO A GREATER ACCUMULATION OF UNREPAIRED DAMAGE THAT DIRECTS TUMOR CELLS TOWARDS APOPTOSIS.

SEVERAL TECHNIQUES HAVE BEEN PROPOSED TO STUDY BRCANESS. AMONG THEM: CONVENTIONAL IMMUNOHISTOCHEMISTRY TECHNIQUES WITH SPECIFIC MARKERS; PYROSEQUENCING TO DETERMINE THE METHYLATION DEGREE OF THE BRCA1 PROMOTER; MULTIPLEX LIGATION-DEPENDANT PROBE AMPLIFICATION (MLPA), COMPARATIVE GENOME HYBRIDIZATION, QUANTITATIVE REAL-TIME PCR AND, MORE RECENTLY, MIRNAS AND TUMOR CIRCULATING DNA STUDY THROUGH DIGITAL DROPLET PCR (DDPCR).

KEYWORDS: BREAST CANCER, TNBC, BRCANESS, HRD, IPARP, BRCA1, BRCA2.

PALABRAS CLAVE: CÁNCER DE MAMA, TNBC, BRCANESS, HRD, IPARP, BRCA1, BRCA2.

Epidemiology and classification of breast cancer

Breast cancer is the neoplasm of highest incidence, prevalence and mortality among women in developed countries (30% of the total) (1). In Spain 26000 new cases are diagnosed annually, 50% of them in women between 50 and 69 years. This age range is covered by early diagnosis strategies (mammography). The remaining 50% is subdivided as follows: 25% in women below 50 years and 25% in women over 70 years.

Familial aggregation of breast cancer, as well as its presentation within genetic cancer predisposition syndromes, is more common among patients below 50 years. In these patients, the disease is also more aggressive, showing lower survival rates. This correlates with a higher incidence of triple negative breast cancer (TNBC) in this population. It is also related to late diagnosis, usually after the patient's consulting (lump in 71.6% of Spanish cases) (2).

The mortality rate of TNBC in women over 70 years is higher than in women between 50 and 69 years. This is related to the lack of a specific screening and the occurrence of frequent comorbidities in addition to the tumor process. As in other malignant processes, unmodifiable risk factors are age, sex, race and family and personal history of the disease. In the case of breast cancer, early menarche and late menopause are included. Modifiable factors include: use of oral contraceptives, alcohol, western diet, number of pregnancies and breastfeeding (3).

Breast carcinomas are a heterogeneous group of lesions previously classified solely based upon histological features and staining pattern for estrogen receptor (ER), progesterone receptor (PR) and HER2 (protein from the superfamily of the epidermal growth factor). Most of these tumors are ER-positive and PR-positive, with HER2 usually expressed. This is associated with good prognosis. RE is positive in 65% of tumors in women under 50 years, while 60% are positive for RP. Positivity for hormone receptors and HER2 overexpression appear in 6% of cases. This is associated with poor prognosis and early resistance to endocrine therapy alone (4). Tumors negative for these three markers, TNBC, represent 15% of all cases. Both overall and disease-free survival are low in these cases. Patients respond quickly to classical cytostatic agents used as first line treatments, but they develop visceral metastases early (5).

The histopathological classification does not describe optimally mammary carcinomas, neither their characteristics nor their behavior. Therefore, a molecular classification of

breast cancer has been developed, complementary to the traditional classification. The following lesion types can be distinguish according to this classification: Luminal A, luminal B, with HER2 overexpression, basal profile and claudin-low. The characteristics of each group are shown in table 1.

Hereditary breast-ovarian cancer syndromes affect several relatives, young at the time of diagnosis, bilateral breast cancer, synchronous breast and ovarian cancer or metachronous in one or more patients in the family or breast cancer in men. It shows an autosomal dominant inheritance pattern. The most commonly implicated genes are listed in table 2 (11).

Molecular subtype	Prevalence (%)	Genetic profile	Histophatologic characteristics	Treatment / Prognosis
Luminal A	40	RE strong positive, RP+/–, HER2–, increase in expression of luminal cytokeratin yand genes related to RE, Ki67 low.	Low grade, RE+	GOOD Endocrine (chemotherapy only in selected patients)
Luminal B	20	RE weak positive, RP+/–, HER2+/– increase in expression of genes related to proliferation, Ki67 high.	High grade, RE+	INTERMEDIATE Endocrine + Chemotherapy +/– anti HER2
Overexpression HER2	10-15	HER2–, RE–, RP–.	High grade, +/– apocrine characteristics	POOR Chemotherapy + anti HER2
Basal Type (basal types 1 and 2)	15-20	RE, RP y HER2–, cytokeratin 5/6 + EGFR+/–, increase in expression of cytokeratin.	High grade, high mitotic index, necrosis, lymphocyte inflitration	POOR Chemotherapy
Claudin low (mesenquimal type y mesenquimal stem cell type)	12-14	RE, RP y HER2–, low Claudin 3/4/7, vimentine+, low E-cadherine, low expression of proliferation genes.	High expression of markers of epithelium-mesenchyme transformation	POOR Chemotherapy

▲ **Table 1.**

Familial breast cancer. Hereditary breast-ovarian cancer syndromes.

Between 5-10% of breast cancer cases are included within hereditary syndromes with a Mendelian inheritance pattern (6). Moreover, up to 15% of patients show familial aggregation without any identified syndrome establishing the features and genetic basis. The mutations described in patients with hereditary syndromes are distributed as follows (7):

- 20-40% BRCA1 (Breast Cancer 1)
- 10-30% BRCA2 (Breast Cancer 2)
- 7% TP53, PTEN, ATM, CHEK2, BRIP1
- Other cases. Unidentified predisposing genes.

Familial breast cancer is defined on the basis of coalescence of cases without any autosomal dominant pattern. There is a weaker, poorly defined, genetic predisposition. This is likely the result of the interaction of low penetrance genes together with the effect of environmental factors. Among the risk factors are: maternal breast cancer, early menarche and late menopause, old age, age at first birth, use of contraceptives, atypical hyperplasia, diet and exposure to carcinogens.

BRCA1 and BRCA2

BRCA1 and BRCA2 are proteins involved in homologous recombination repair (HRR) of the double-strand breaks (DSB) in DNA. Therefore, the loss of function of any of them reduces the ability to maintain the integrity of the DNA, increases genomic instability and favors the development of neoplasms. Failure in BRCA favors the activation of alternative repair pathways, less effective and recently related to the enhancement of tumor development.

BRCA1 was cloned in 1994, is located in 17q21 and has 24 exons. Among the functions of the encoded protein are: DNA repair via HRR, cell cycle control, apoptosis, genomic instability, transcriptional activation and tumorigenesis. Mutations described in BRCA1 are concentrated in three regions, the RING domain at the N-terminus (exons 2-7), 11-13 exons region and C terminus of the protein (exons 16-21) (7).

BRCA2 was also identified in 1994. It has 27 exons and is located in 13q12. Although there are some similarities in the coding region of both genes, there is no substantial homology between BRCA1 and BRCA2. Mutations of the terminal

Gen	Síndrome	Otras neoplasias asociadas
High penetrancy		
BRCA1	Hereditary Breast-Ovarian cancer Syndrome	Ovary, pancreas
BRCA2	Hereditary Breast-Ovarian cancer Syndrome	Ovary, pancreas, prostate
p53	Sd. Li-Fraumeni	Sarcoma, osteosarcoma, cerebral tumor, suprarenal carcinoma, leukemia, colon cancer
PTEN	Cowden Disease	Tyroid gland, endometrium, genitourinary
STK11/LKB1	Peutz-Jeghers Syndrome	Small intestine, colorectal, uterus, testicle, ovary
CDH1	Hereditary diffuse gastric carcinoma	Lobular breast cancer, diffuse gastric
Low-moderate penetrancy		
ATM	Ataxia telangiectasia	—
CHEK2	Li-Fraumeni Variant	—
BRIP1	Fanconi Anemia	—
PALB2	No associated syndrom	—

▲ **Table 2**.

end of BRCA2 are the most common with a known clinical significance. Its basic function is to repair DNA damage (7).

More than three thousand variants in the BRCA1 and BRCA2 genes have been registered in Breast Cancer Information Database. The impact of many of these variants on the protein function remains unknown. Therefore they cannot be defined as mutations or polymorphisms and are known as variants of unknown significance (VUS). This occurs in up to 25% of families with hereditary breast-ovarian cancer syndrome.

BRCA1 and BRCA2 are considered tumor suppressor genes (TSG). They are involved in the repair of DSBs by HR interacting with RAD51. Once the DNA damage is detected, ATM or ATR proteins act as sensors. Then, BRCA1 is associated with RAD51, locates itself over the damaged region and is phosphorylated and ready to act. BRCA2 is a mediator further down the same pathway, also associating with RAD51. As previously said, the main function of BRCA2 is damage repair by HR. However, BRCA1 has this and other roles. It belongs to a group of proteins responsible for genome surveillance (BRCA1 Associated genome surveillance complex, BASC) (8, 9) together with others like MSH2, MSH6, MLH1, ATM, BLM. The increase in BRCA1 and BRCA2 levels during S phase has been associated with the maintenance of genomic stability in this phase (7, 8).

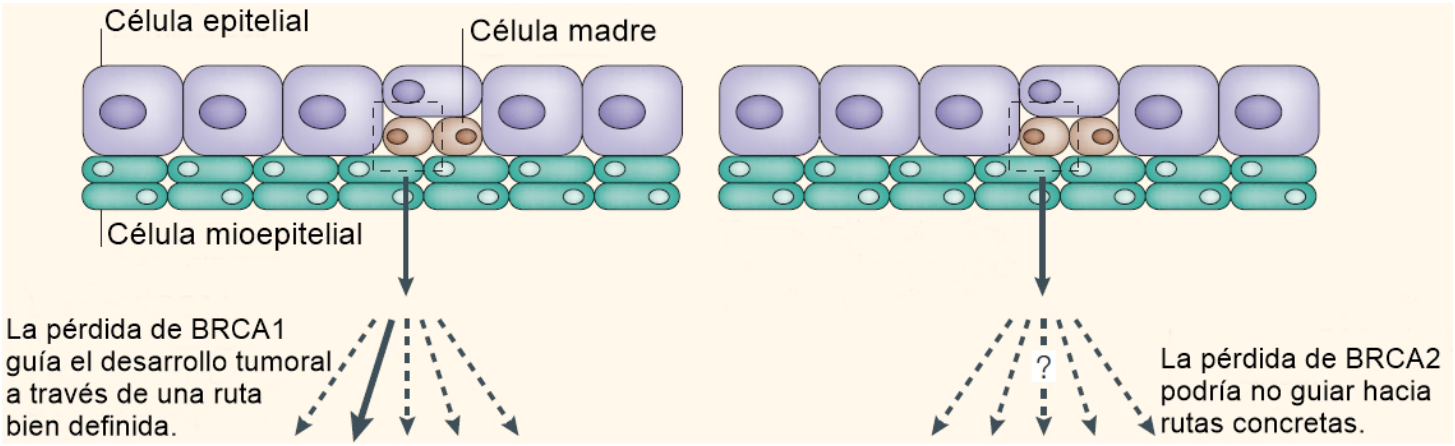
Carcinomas developed in BRCA1/2 mutation carriers in germline present increased genomic instability secondary to DNA repair deficit. This point is considered the origin of the neoplasm and promoter of its development. However, in normal cells, accumulation of errors in DNA guides to cell cycle arrest or cell death via apoptosis. Thus, tumor development requires mutations in one of these TSG and alterations in the cell cycle regulatory mechanisms. In this regard, it has been found that mutations in TP53 and AURORA 2 and amplifications in c-Myc are more frequent in BRCA deficient mammary carcinomas (10).

Carcinomas are thought to develop from mammary epithelial stem cells. In patients with BRCA1 mutations in germline, metabolic pathways that form basal neoplasms are enhanced. In the case of BRCA2, no predominant activation of any particular metabolic pathway has been described. Immunohistochemistry profile of these tumors is variable, with a proportion of ER positive similar to that of sporadic carcinomas.

Molecular classification of TNBCs

Among the TNBCs, the first molecular classification described two subtypes: claudin low and basal type. The former is characterized by low expression of the constituent proteins of the tight junctions (claudins 3, 4 and 7) and E-cadherin. Active transcription of genes related to immune response such as CD4 and CD79a is typical. Basal type carcinomas are characterized by overexpression of basal markers (cytokeratin 5 and 8), increased expression of genes related to cell proliferation, altered DNA damage repair and cell cycle checkpoints mechanisms. The overlap rate of TNBCs and basal type carcinomas varies between 60-90% (2) (15).

Lehmann *et al.* (12) and Bertucci *et al.* (13) agree that TNBCs are the group of mammary tumors with the most heterogeneous transcriptional pattern. These authors state that the initial classification in claudin-low and basal subtypes did not faithfully reflect the types existing in this disease and



Fenotipo BRCA1		Fenotipo BRCA2
Basal	Rasgos específicos de desarrollo tumoral en lesiones con defecto en la vía BRCA	¿? Sin subtipo específico
RE negativo		Proporción de RE positivo como esporádicos
Expresión EGFR		-
Infiltración linfocítica		-
Amplificación c-MYC		¿? Amplificación c-MYC
Mutaciones TP53	Rasgos que manifiestan defecto en la capacidad reparadora de DNA	Mutaciones TP53
Pérdida de RAD51-focus		Pérdida de RAD51-focus
Inestabilidad genómica extrema		Inestabilidad genómica extrema
Sensible a agentes alquilantes		Sensible a agentes alquilantes

▲ **Figure 1**. *Modified from Turner N, Tutt A, Ashworth A. Opinion: Hallmarks of 'BRCAness' in sporadic cancers. Nature Reviews Cancer. 2004;4(10):814-819.*

- *Epithelial cell, myoepithelial cell, stem cell. The loss of BRCA1 leads the tumor development through a well-defined path. The loss of BRCA2 could not lead to definite pathways.*
- *Phenotype BRCA1.*
  Specific characteristics of tumor development in lesions with failure in BRCA pathway:
  - *Basal.*
  - *RE negative.*
  - *EGFR expression.*
  - *Lymphocyte infiltration.*
  - *c-MYC amplification.*

  Characteristics showing a failure in DNA repairing ability:
  - *TP53 mutations.*
  - *Loss of RAD51-focus.*
  - *Extreme genomic instability.*
  - *Responsive to alkylating agents.*
- *Phenotype BRCA2.*
  Specific characteristics of tumor development in lesions with failure in BRCA pathway:
  - *Without specific subtype.*
  - *Proportion of RE positive as sporadic.*
  - *c-MYC amplification.*

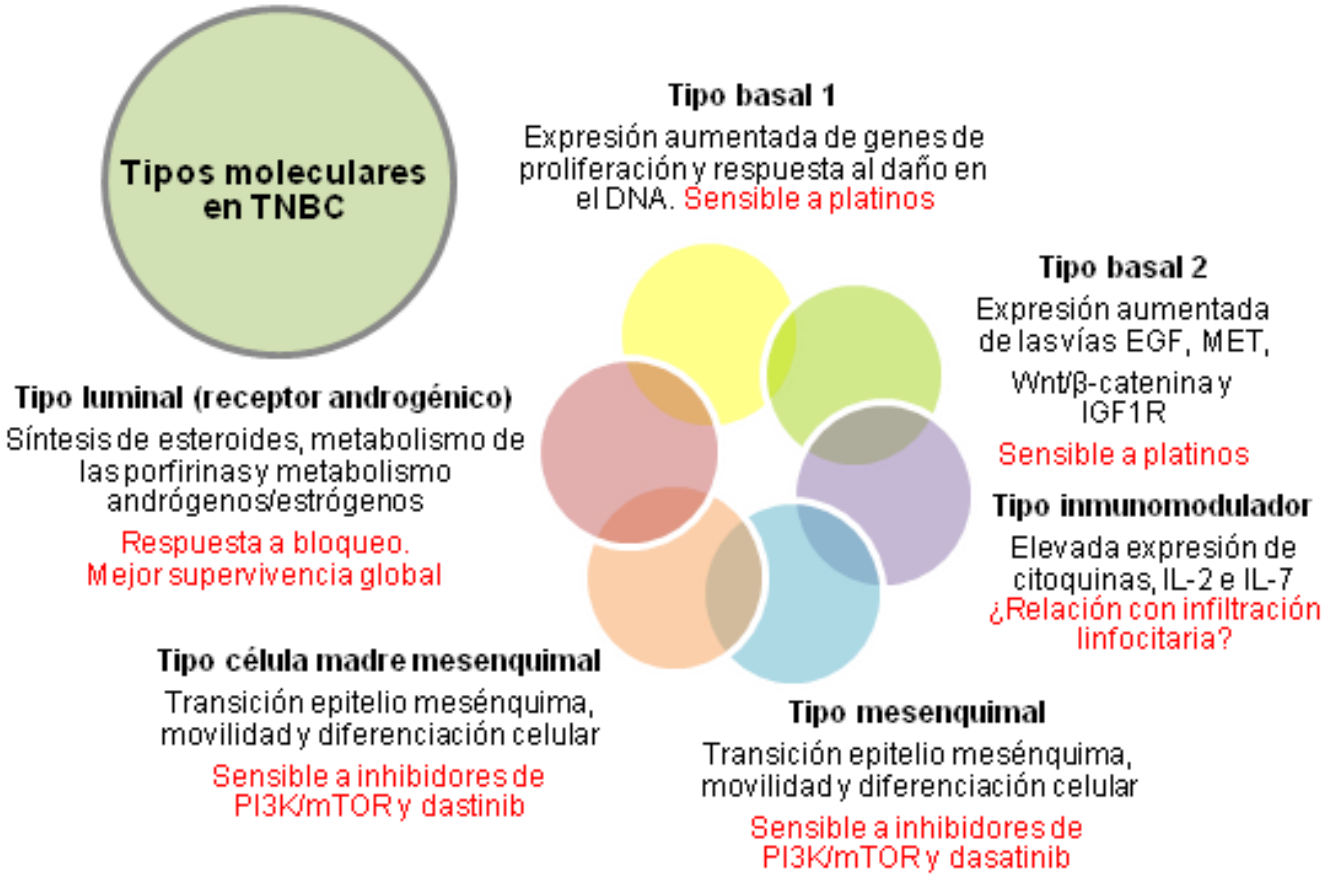
  Characteristics showing a failure in DNA repairing ability:
  - *TP53 mutations.*
  - *Loss of RAD51-focus.*
  - *Extreme genomic instability.*
  - *Responsive to alkylating agents.*

added six new subtypes: basal 1, basal 2 immunomodulator group, mesenchymal, mesenchymal stem cell and luminal (androgen receptor) (15).

Definition of BRCAness in Breast Cancer

Tumors from patients with BRCA1 mutations in germ line share common characteristics with basal type lesions. In fact, up to 75% of lesions in these patients belong to this molecular subtype. Alterations in the BRCA1 pathway in basal carcinomas are mostly associated, as previously stated, with the HR of DSBs in DNA. This is relevant considering that detecting this error warrants the use of specific agents (Alkylating agents, PARP inhibitors). Defects in this repairing pathway, BRCA, gives name to a specific phenotype of breast carcinoma, BRCAness. The correct identification of this group of patients is unavoidable for a precise classification of the disease and adequate treatment. There is heterogeneity between breast carcinomas. In the same way, TNBC are not equal (12, 15).

BRCAness is thus defined by a deficit in DSBs reparation of DNA by HR that leads to genomic instability and development of carcinomas. The origin of the alteration in the BRCA pathway may be diverse, but has similar consequences (poor prognosis due to early recurrence after conventional



▲ **Figure 2**. *Molecular types of TNBC.*

- **Type basal 1:** *increased expression of proliferation and DNA damage response genes. Responsive to platins.*
- **Type basal 2:** *increased expression of the pathways EGF, MET, Wnt/β-catenine and IGF1R. Responsive to platins.*
- **Type immunomodulator:** *increased expression of cytokines, IL-2 and IL-7. Related to lymphocyte infiltration?*
- **Type mesenchyme:** *transition epithelium-mesemchyme, mobility and cell differentiation. Responsive to PI3K/mTOR inhibitors and dasatinib.*
- **Type mesenchyme stem cell:** *transition epithelium-mesemchyme, mobility and cell differentiation. Responsive to PI3K/mTOR inhibitors and dasatinib.*
- **Type luminal (androgenic receptor):** *steroid synthesis, porphyrin metabolism and androgens/oestrogens metabolism. Responsiv to blockage. Better overall survival.*

treatment). In hereditary syndromes, BRCAness is secondary to the status of BRCA1/2 mutations carrier. However, BRCAness in sporadic carcinomas may appear as a consequence of: somatic mutations of BRCA1/2 (uncommon), methylation of BRCA1 promoter (11-14%), overexpression of transcriptional repressors of BRCA1 (ID4), loss of function of BARD1, methylation of FANCF (17%), amplification of EMSY (13%), specific alterations in the number of copies, loss of PTEN alone or combined with RAD17 and RAD50, mutations in TP53, etc. (3, 14)

TP53 mutations are frequent (52.4%) in tumors from carriers of pathological changes in BRCA1 (3). They are also common in sporadic malignancies that display positive BRCA1ness phenotype (16). Modifications of TP53 are secondary to defective DNA repair.

Functionality studies of RAD51 may show abnormalities in HR and support the diagnosis of BRCAness. In the same way, expression of PARP1 can be measured in these cases and in BRCA1/2 carriers. PARP1 is overexpressed in 91% (3) of these patients, showing the importance of this pathway and the vast potential activity of its pharmacological inhibitors.

FANCF codifies a protein that forms complexes in the nucleus and its alteration prevents correct DNA repair. It has been related to the development of Fanconi anemia and breast cancer (10). Patients who are recessive homozygous for mutations in this gene show a high sensitivity to alkylating agents.

The methylation of BRCA2's promoter has not been described in breast carcinomas (3, 10) and it is considered as an exceptional event in ovarian carcinomas.

One of the mechanisms described to explain the silencing of BRCA2 in sporadic malignancies is the amplification of EMSY (10). EMSY is able to interact with the protein domain codified by exon 3 of BRCA2, which is related to transcriptional regulation. Besides, this protein interacts with other proteins that are part of chromatin remodelling complexes.

A lower expression of BRCA2 is observed when EMSY is over-expressed. It is still unknown if this is enough to convert cells into BRCA2-deficient. If so, BRCA2ness positive tumor cells copying the phenotype of other BRCA2 mutants in germ line would appear as a result of the amplification of EMSY.

Altered expression of APE1 and aberrant methylation of MGMT are also emerging as notable processes within the TNBC and as potential markers of BRCAness phenotype. Fumagalli *et al.* found this epigenetic alteration in more than 60% of all the TNBC they examined (17).

BRCAness diagnosis

BRCAness phenotype can be studied by using several techniques, including: compared genomic hybridization (CGH), multiplex ligation-dependent probe amplification (MLPA), real-time polymerase chain reaction (qPCR) and immunohistochemistry. Recently, miRNA analysis and circulating tumor DNA with ddPCR (digital doplet PCR) have been added to this group of techniques. All of them seek to reveal a characteristic phenotype of BRCA1/BRCA2-defective cells, with a deficit in HR. Moreover, the analysis of specific markers (TP53 mutations, BRCA methylation...) can be completed with conventional techniques.

There is still no standardized technique to characterize these lesions, but efforts are being made to make it soon available in clinical practice (14).

BRCAness and breast cancer treatment

Cells have multiple mechanisms to repair DNA, with the following five being the main types: homologous recombination, base excision repair (BER), nucleotide excision repair, mismatch repair and direct repair (18). If one of these ways fails or is deficient, the damage is detected and either the progression of the cell cycle is stopped or apoptosis is promoted. However, occasionally, these alterations cause genome instability. In this situation, mutations may appear and, when not corrected, they can promote tumor development. This is the case of the already discussed alterations that lead to BRCAness in breast cancer.

Many antineoplastic agents cause direct lethal damage in the DNA of tumor cells. Resistance to treatment can be determined by the efficacy of repair mechanisms to amend the alterations caused. For some time now it is possible to modulate the activity of DNA repair mechanisms to create an imbalance in favor of cell damage and death. This is the case of PARP inhibitors.

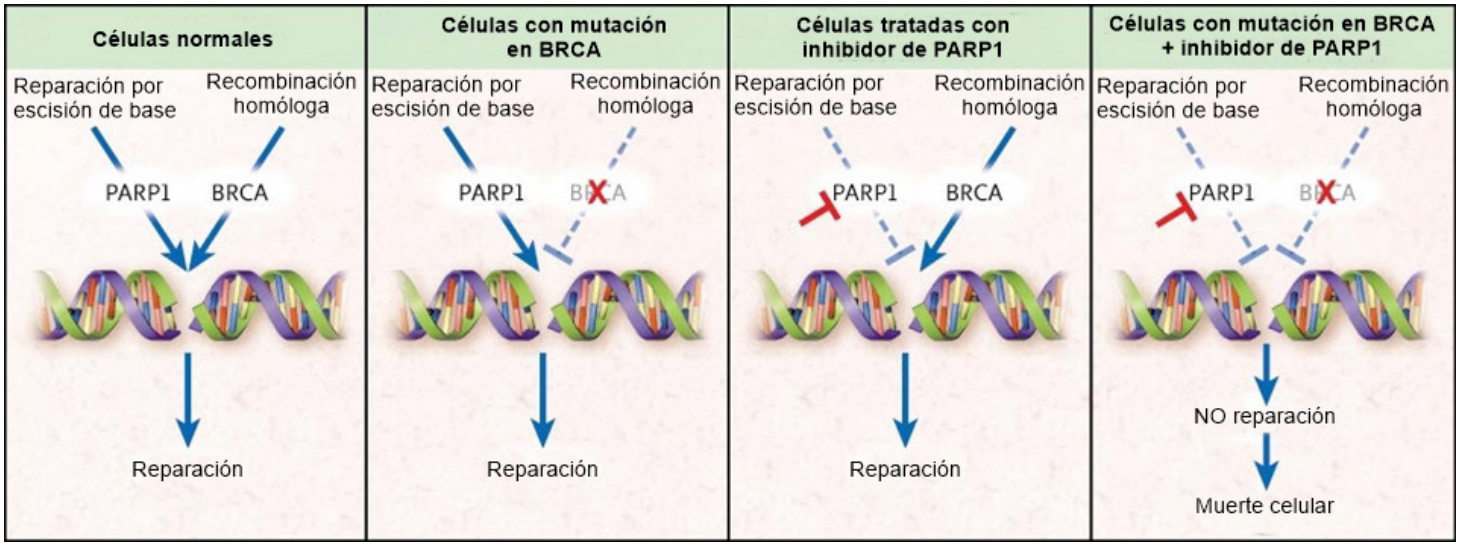
Poly(ADP-ribose)polymerases (PARP) are a highly conserved family of enzymes involved in base excision repair of single-strand breaks of DNA. PARP acts a signal and promotes the recruiting of other proteins (XRCC1, DNA ligase III and DNA polymerase B) that form the BER complex. Once agglutinated, the affinity of PARP-1 for DNA decreases, the chromatin condenses and the repair is possible (14) (18).

Many chemotherapy agents, such as alkylating agents or camptothecins, produce single-strand breaks in the DNA. Because of that, PARP inhibition was originally suggested as a possible chemotherapy enhancer (19). The combination of alkylating agents and PAPR inhibitors (PARPi) has been

▼ **Figure 3**. *Modified from Iglehart J, Silver D. Synthetic Lethality —A New Direction in Cancer—. Drug Development. New England Journal of Medicine. 2009;361(2):189-191.*

- *Normal cells.*
- *Cells with BRCA mutation.*
- *Cells treated with PARP1 inhibitor.*
- *Cells with BRCA mutation+PARP1 inhibitor.*

Base excision repair.
Homologous recombination.
Repair/No repair → Cell death.



tested. A phase II clinical trial (O'Shaughnessy *et al.*) showed a better response and global survival in patients with metastatic TNBC when confronting the arms: carboplatin+gemcitabine versus carboplatin+gemcitabine+iniparib (PARPi) (20).

In 2005 it was verified that cells deficient in HR are hypersensitive to base excision repair inhibition. When using iPARP, cell death was increased. Several mechanisms have been suggested to explain this cytotoxicity, but none of them has been proved conclusive (23).

Single-strand breaks' blockage by PARP leads to the onset of double-strand breaks in the replication fork. In cells deficient in HR, like the BRCAness positive ones, this damage cannot be repaired. Then synthetic lethality happens and, consequently, cell death. On the contrary, PARPi administration in BRCA-competent cells does not produce the same response. In the event of double-strand breaks formation, these lesions are repaired and the cell cycle continues its course.

Auto-ribosylation of PARP1 is thought to be necessary for its release from DNA. iPARP could catch PARP1 before this step and promote aggregates formation attached to DNA. Under these circumstances, replication could be hampered. In cells with HR failure, this point could be precluded. Therefore, cell death is increased in BRCA-deficient cells.

Olaparib was the first iPARP tested alone. It was used in the treatment of advanced breast, ovarian, lung and prostate cancers in patients with BRCA1/2 mutations in germ line (22). The results were good, with a response rate of 41% for breast cancer, and manageable side effects.

The use of iPARP in TNBC and sporadic ovarian carcinoma has led to uneven results (21, 23). This has been related to the heterogeneity of these malignancies, since not all of them were BRCA deficient and, therefore, probably not equally responsive to the antitumor effect of iPARP. It is necessary to identify BRCA positivity before starting the treatment with BER inhibitors.

Another hypothesis has been tested for sporadic breast cancer more recently: combining homologous recombination inhibitor drugs with iPARP. In this way, a response similar to BRCA positive cases is expected. Examples of this are the combinations: CDK1+iPARP / PI3K+iPARP / Histone deacetylases+iPARP.

Resistances to iPARP in BRCAness positive carcinomas have been described. They have been related to: BRCA's function recovery due to secondary mutations, loss of 53BP1 expression, residual activity of BRCA mutant proteins, up-regulation of ABC membrane transporting protein (ATP-Binding-Cassette) and aberrant expression and/or activity of PARP (3, 23).

Besides iPARP, the role of APE1 endonuclease inhibitors (iAPE) in breast cancer is being also studied (24). Its validity as an antitumor agent is promising. The development of small molecule-iAPE1 is an area of intense research. Likewise, it has been verified that the repression of APE1 enhances the sensitivity to temozolomide, a drug already used in gliomas with methylated MGMT.

▲ **Figure 4**. *Resistance to iPARP.*

- *Secondary mutations in BRCA1/2.*
- *BRCA1/2 residual activity.*
- *ABC up regulation.*
- *PARP expression alteration/function alteration.*
- *Loss of expression of 53BP1.*

Conclusions

The molecular classification of breast cancer and the description of the BRCAness phenotype have opened the gates to hope in TNBC. Enhancing DNA damage in HR deficient cells has proved to be an excellent antitumor strategy. Although it is still not possible to access these drugs outside the trial, iPARP or iAPE, either in monotherapy or with other classic agents, will be a promising alternative in clinical practice in the short term. Therefore, it is vital to quickly standardize a BRCAness diagnostic method. It is also necessary to deeply study the emerging resistance mechanisms to iPARP.

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