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The Fanconi anaemia/BRCA pathway and cancer susceptibility. Searching for new therapeutic targets

M.ª José García · Javier Benítez

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Abstract Breast cancer is one of the most frequent cancers in the world. The majority of cases are sporadic but around 15% show some type of familial aggregation and about 5% exhibit a clear hereditary pattern. Common and rare low–moderate-penetrance genes, and high-penetrance genes are thought to explain the genetic susceptibility to the disease. Only around 20% of the inherited risk to breast cancer is explained by germline mutations in the known high-penetrance susceptibility genes *BRCA1* and *BRCA2*. Mutations in genes such as *TP53* and *PTEN* have also been linked with high risk for breast cancer within specific cancer syndromes and rare germline variants in genes such as *CHEK2* and *ATM* have been found to confer modest risk to breast cancer. However, we can say that less than 30% of familial risk of breast cancer is due to known genes. Identification in 2002 of the Fanconi anaemia (FA) gene *FANCD1* as *BRCA2* and recent studies indicating that heterozygous mutations in *FANCN/PALB2* and *FANCJ/ BRIP1* predispose to breast cancer have emphasised an important connection between the FA and BRCA pathway. Here we review the emerging DNA-damage response network consisting of FA and BRCA proteins, summarise what is currently known about the direct involvement of these molecules in breast cancer susceptibility and discuss the prospect offered by this pathway in order to identify more breast cancer related genes. We finally present the current stage of therapeutic options specifically targeting the FA/BRCA pathway and summarise the challenges this field encounters.

Keywords Fanconi anaemia · Breast cancer susceptibility genes · *BRCA1* · *BRCA2* · Targeted therapy

Introduction

Breast cancer is one of the most frequent cancers in the world. About 5% of Spanish women will develop this type of cancer during their life and it represents 16,000 new cases every year [1]. Different factors have been associated to breast cancer susceptibility, genetic factors being the most important as twin studies or large epidemiological studies suggest.

The majority of breast cancers (80%) are sporadic and they can be currently explained by a polygenic model in which the interaction of different genes (plus environmental factors) would be necessary to develop the disease. These genes are known as common low-penetrance genes (LPG) and although individually they confer a small risk (<1.5 fold), the fact that they are very frequent in the general population (>5%) could make a considerable contribution to tumour development [2]. During 2007, different consortia have described some LPGs, each of them conferring a small risk that ranges in general between 1.1- and 1.3-fold [3–5]. About 15% of breast cancer cases present

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M.J. García · J. Benítez (✉)
Group of Human Genetics,
Human Cancer Genetics Program,
Spanish National Cancer Centre (CNIO)
and Centre for Biomedical Research on Rare Diseases (CIBERER),
Instituto de Salud Carlos III,
C/ Melchor Fernández Almagro 3,
ES-28029 Madrid, Spain
e-mail: jbenitez@cnio.es

Table 1 Percentage of familial breast cancer risk explained by the currently known different types of susceptibility genes

Gene	Type	MAF	RR/allele	Familial risk (%)
FGFR2	cLPG	38%	1.26	2
TNRC9	cLPG	25%	1.20	1
MAP3K1	cLPG	28%	1.13	0.5
LSP1	cLPG	30%	1.07	0.2
8q	cLPG	40%	1.08	0.3
2q	cLPG	50%	1.21	1.5
CASP8	cLPG	13%	0.89	0.2
ATM	rMPG	0.3%	2.30	0.7
CHEK2	rMPG	0.5%	2.20	1
BRIP1	rMPG	0.1%	2.10	0.1
PALB2	rMPG	0.1%	2.30	0.2
BRCA1	rHPG	1/1,500	20	10
BRCA2	rHPG	1/1,500	12	10
TP53	rHPG	1/5,000	>50	1
PTEN	rHPG	1/25,000	10	1

familial aggregation and tumours in these families can be explained by rare LPGs, that is, rare variants that are present in a low percentage of population (<1%) conferring a low–moderate risk of breast cancer (2–4 fold) [2]. It is likely that they make little impact on the disease but the number of them could be higher than hundreds, accounting for an important proportion of the familial risk in breast cancer. *CHEK2* and *ATM* are two of these genes that confer risk near to 2.5 in carriers of specific mutations [6–8]. It has also been estimated that common and rare LPG would explain part of the breast cancer familial aggregation (Table 1). Finally, about 5% of breast cancers are hereditary and they would be explained by rare high-penetrance genes, that is, genes that confer an increased risk for cancer development (>5 fold). *BRCA1* and *BRCA2* are the most important and well known breast cancer susceptibility genes although we currently know that they only explain about 20–25% of hereditary cases [9, 10]. Other genes such as *P53*, *PTEN* or *STK11* are also associated to breast cancer susceptibility but they would explain a small percentage of cases, less than 1% each. Therefore, we can say that less than 30% of familial risk of breast cancer is due to known genes (Table 1).

The BRCA genes

BRCA1 and *BRCA2* were identified by linkage studies in 1994 and 1995 and are associated to familial breast and ovarian cancer. In these families gene transmission follows a dominant model and those women carrying an alteration in any of these genes present a high probability of developing cancer. The incidence is estimated to be 1/1500, which implies that about 6500 women in our country older than 30 years of age carry a mutation in *BRCA1* or *BRCA2*. The percentage of high-risk families due to mutation in any of these genes was initially overestimated because the first studies were done in families with many affected members [11]. However, we and others have demonstrated that only 25% of the high-risk families assisted in Genetic Counselling Units are explained by mutation in any of the two genes [9, 10]. The same occurs with the penetrance or risk that a carrier develops breast or ovarian cancer during their lifetime. This risk has nowadays been estimated to be around 50% and 20% respectively at age 70 years [12–14].

Both genes play an important role in DNA repair (double-strand DNA), replication and cell cycle control. In addition, *BRCA1* also participates in transcriptional regulation and chromatin modification [15, 16]. Regarding the DSB repair function, it has been proposed that upon DNA damage and subsequent activation of H2AX by ATM, a signal transmission pathway that involves the new recently described genes *RAP80* and *CCDC98* is initiated, both genes being crucial for the translocation of BRCA1 to the DNA damage foci [17–19]. DNA damage would also activate BRCA2, its main role consisting of transporting RAD51 to the nucleus and in cooperation with other proteins contributing to the formation of the DNA repair foci [20].

Fanconi anaemia

Fanconi anaemia (FA) is a rare genetic disease (1/200,000–1/400,000 new born) characterised by bone marrow failure, congenital abnormalities and a high risk of malignancies, both haematological and solid tumours [21]. It is a recessive disease, which means that both parents are heterozygous for a mutated gene while the affected child is homozygous. At a cellular level, FA is associated with a high degree of genomic instability such as chromosomal fragili-

Table 2 FA genes and related malignancies

Gene	Incidence in FA patients* (%)	Chromosomal location	Functional subgroup*	Principal cancers in biallelic mutation carriers (phenotype of FA patients)	Cancer susceptibility in monoallelic mutation carriers
FANCA	66	16q24.3	Group 1	Classic FA: AML, head and neck SCC, anogenital tumours	No current evidence
FANCB	~2	Xp22.31	Group 1	Classic FA: AML, head and neck SCC, anogenital tumours	No current evidence
FANCC	10	9q22.3	Group 1	Classic FA: AML, head and neck SCC, anogenital tumours	No current evidence
FANCD1/BRCA2	~2	13q12-13	Group 3	Aggressive FA: childhood solid tumours (Wilms tumour and medulloblastoma)	Breast and ovarian cancer (high-risk)
FANCD2	~2	3p25.3	Group 2	Classic FA: AML, head and neck SCC, anogenital tumours	No current evidence
FANCE	~2	6p21.3	Group 1	Classic FA: AML, head and neck SCC, anogenital tumours	No current evidence
FANCF	~2	11p15	Group 1	Classic FA: AML, head and neck SCC anogenital tumours	No current evidence
FANCG	9	9p13	Group 1	Classic FA: AML, head and neck SCC, anogenital tumours	No current evidence
FANCI	~2	15q26.1	Group 2	Classic FA: AML, head and neck SCC, anogenital tumours	No current evidence
FANCJ/BRIP1	~2	17q22-24	Group 3	Classic FA: AML, head and neck SCC, anogenital tumours	Breast cancer (low–moderate risk)
FANCL	<2	2p16.1	Group 1	Classic FA: AML, head and neck SCC, anogenital tumours	Unknown
FANCM	<2	14q21.3	Group 1	Classic FA: AML, head and neck SCC, anogenital tumours	Unknown
FANCN/PALB2	~2	16p12.1	Group 3	Aggressive FA: childhood solid tumours (Wilms tumour and medulloblastoma)	Breast cancer (low–moderate risk)

*From W. Wang (2007). Group 1: Form the Core complex that monoubiquitinates FAND2/FANCD1; Group 2: Form the "ID" complex ; activated upon monoubiquitination; Group 3: Molecules acting downstream FANCD2 ubiquitination; crosstalk with other DNA-repair proteins

ty and chromatid interchanges [22]. FA cells are highly sensitive to DNA crosslinking drugs, including mitomycin C (MMC), cisplatin or diepoxybutane [23]. This hallmark is currently used as a clinical diagnostic test and implies a defect in the DNA repair pathway due to the germinal genetic alteration. FA is a heterogeneous genetic disorder and comprises at least 13 complementation groups (A, B, C, D1, D2, E, F, G, I, J, L, M and N), each of them associated with a specific gene named *FANCA-FANCN* [24] (Table 2). *FANCA* is the most common mutated gene worldwide (66% of cases), followed by *FANCC* and *FANCG*, each one of them explaining about 10% of cases [24]. However there are population-specific differences due to founder mutations, as it happens with the Spanish gypsies or Ashkenazi Jews that present a single specific mutation in *FANCA* and *FANCC* respectively [25, 26].

Discovery of the first gene (*FANCC*) was possible by somatic cell hybridisation studies using different complementation groups. Cells from a complementation group C patient were transfected with a cDNA expression library and selected in the presence of MMC. Only those cells that received the *FANCC* cDNA were corrected, allowing their growth and later the identification of the gene [27]. The same approach was used in the discovery of four more genes (*FANCA*, G, F and E) but none of them contained domains that could implicate a role in DNA repair. Early data suggested that the five proteins could form a nuclear complex that was termed the FA core complex [28]. This complex is currently known to involve at least 8 genes (including *FANCB*, L and M) that participate in the monoubiquitinisation of two proteins, FANCD2 and FANCI. When *FANCD2* was cloned in 2001, DNA repair domains were identified and this data allowed establishing a link between FA proteins and DNA damage [29]. *FAND2* and *FANCI* are paralogues that interact and work together in response to DNA damage [30]. Although the function is not clear, the ubiquitination could recruit them to the sites of DNA damage, contributing to the formation of the foci together with BRCA1, BRCA2 and other DNA repair players [31] (Fig. 1).

The link between FA/BRCA pathway and breast cancer

In 2002, Howlett et al. [32] identified the *FANCD1* gene as the *BRCA2* gene, establishing an important connection between the FANC genes and breast cancer. Biallelic mutations in the *FANCD1* gene would result in a FA phenotype, while individuals with heterozygous mutations would be associated to familial breast and ovarian cancer. Although

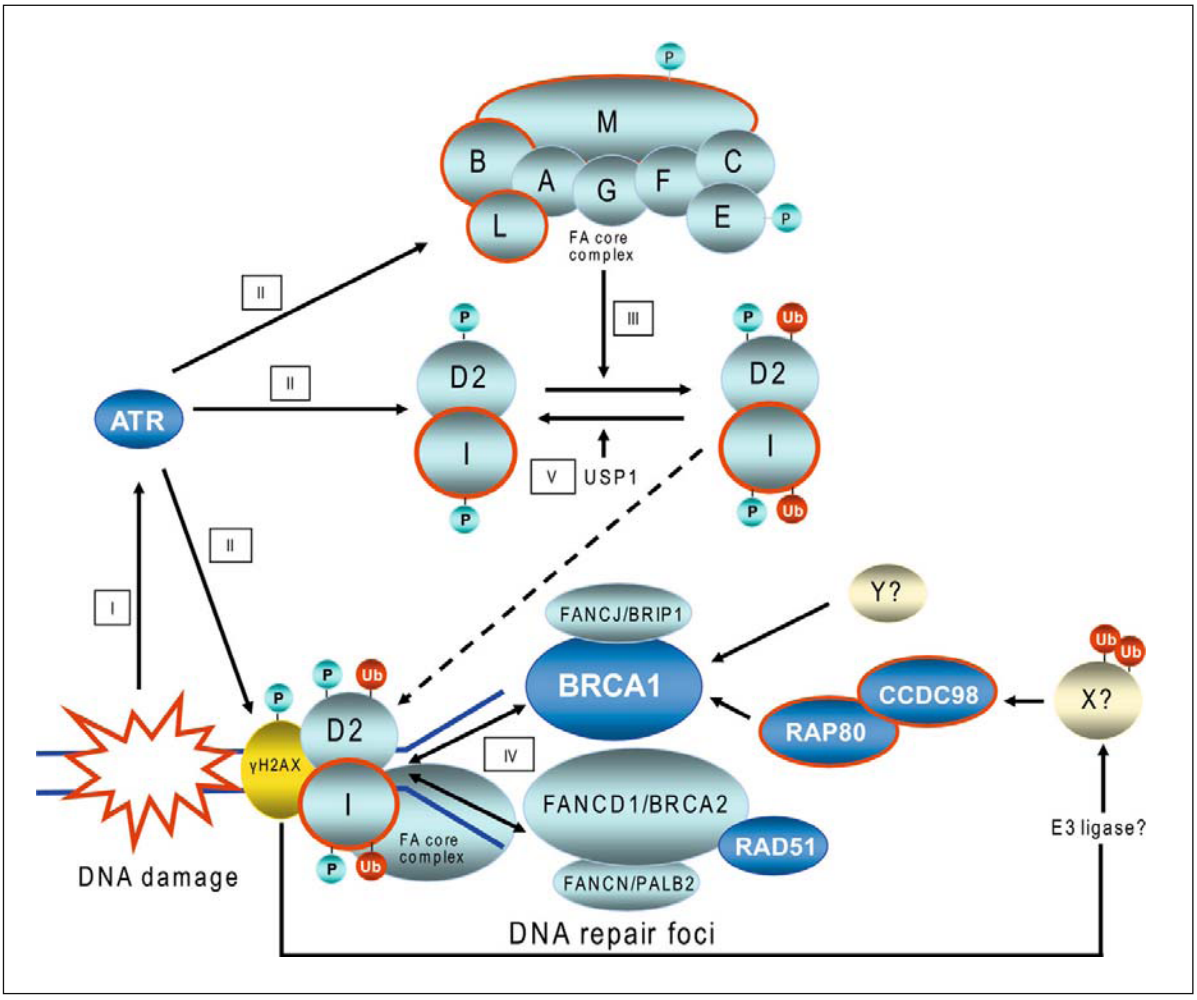

Fig. 1 Schematic representation of the FA/BRCA pathway. This cartoon compiles information from the most recently proposed models for the FA/BRCA pathway [19, 43]. DNA damage and stalled replication fork signal ATR (I) that subsequently phosphorylates the FA core complex (FANCA, B, C, E, F, G and L), the ID FA complex (FANCD2 and FANCI) and the histone H2AX (making γH2AX) (II). Activated FA core complex then monoubiquitinates the ID complex (III) and translocates to sites of DNA damage where at least some of its components such as FANCM might also directly participate in the DNA-repair process (for clarity individual FA core complex proteins are not depicted at the DNA damage foci). Monoubiquitination of the ID complex targets it into the DNA damage foci (discontinued arrow) where it interacts with FANCD1/BRCA2, FANCJ/BRIP1 and FANCN/PALB2 (IV) as well as with other DNA-repair proteins that participate in a DNA-damage response network such as BRCA1 or RAD51. Once the damage is repaired, the enzyme USP1 removes the monoubiquitin from the FA ID complex, inactivating the pathway (V). The newly identified RAP80 and CCDC98 BRCA1-interacting proteins are also depicted: It has been proposed that an unidentified E3 ligase acting downstream H2AX phosphorylation would polyubiquitinate currently unknown substrate proteins (X). This would serve as a signal for RAP80 to bind CCDC98, the RAP80-CCDC98 complex acting upstream BRCA1 regulating its localisation and function after DNA damage. It is likely that proteins other than RAP80 and CCDC98 also participate in this process (Y). Proteins encircled in red are those whose possible role in breast cancer susceptibility has not been tested yet (our group has recently discarded a major implication of FANCB; [41])

infrequent (2%), this subtype of FA is one of the most aggressive and severe forms. In addition to the classical features of the disease, patients of this complementation group present a spectrum of distinct characteristics such as high risk of developing childhood solid tumours, mainly Wilms and brain tumours [33]. These data suggested that FA could be an attractive model to identify more breast cancer susceptibility genes and examine how they function. In 2003 the first study analysing five known genes of the FA core complex in breast cancer families without mutation in *BRCA1* or *BRCA2* (BRCAX families) failed to demonstrate any relation with breast cancer [34]. In 2006, Seal et al. reported that BRIP1, a BRCA1 interacting protein identical to the *FANCJ* gene, was also a breast cancer susceptibility gene that explained about 1% of BRCAX cases [35]. The relative risk for a person carrying a mutation in *BRIP1* is estimated to be around 2. In 2006 a new BRCA2-interacting protein named PALB2 was identified and one year later this gene was found to be mutated in FA individuals, and termed *FANCN* [36–38]. FANCN/PALB2 colocalises

in the nuclear foci with BRCA2, promotes its integration in key nuclear structures and facilitates its recombinant function. This gene explains a small number of FA patients but the phenotype is very similar to *FANCD1 (BRCA2)* with the presence of solid tumours. We can say that almost all FA children with solid tumours can be explained by mutation in these genes [38] (Table 2). A large study comprising nearly 1000 BRCAX patients from UK showed that *PALB2* is a breast cancer susceptibility gene that explains 1% of BRCAX families [39]. The same percentage of familial breast cancer attributable to mutations in *PALB2* has been reported in Canada [40] and recently in Spain [41], whereas a single *PALB2* founder mutation would be responsible for 1% of all breast cancer occurring in the Finnish population [42]. While biallelic inactivation of *PALB2* gives a FA phenotype, individuals with heterozygous mutation present an estimated risk of 2.4 to develop breast cancer. So, both genes, *PALB2* and *BRIP1*, can be considered as moderated risk genes associated to familial breast cancer.

FANCI, the most recently cloned FA gene [30, 31], is a paralogue of *FANCD2* and its potential connection to breast cancer has not been studied yet. The same occurs with *FANCM* and *FANCL* that belong to the FA core complex. In this regard, a comprehensive analysis of *FANCB* in 96 BRCAX families allowed us to rule out a major contribution of this gene to breast cancer susceptibility [41]. Finally, two new genes have been recently identified interacting with BRCA1 [19, 43]. Receptor associated protein 80 (RAP80) and CCDC98 proteins form a complex necessary for an optimal accumulation of BRCA1 to the sites of DNA damage, that is, in the nuclear foci (Fig. 1). Because there are a few FA patients without association to any of the 13 known FA genes, it would be interesting to examine whether any of them is defective in FA and their possible role as breast cancer susceptibility genes.

Therapeutic targets. Current state and future challenges

In addition to the above-mentioned implication of *FANCD1/BRCA2, FANCN/PALB2* and *FANCJ/BRIP1* in familial breast cancer, it is also known that FA gene defects also occur in a variety of sporadic solid tumours developed in the general population (non-FA patients). *FANCC* and *FANCG* have been found to be mutated in pancreatic cancer [44–46] and epigenetic silencing of *FANCF* has been described in ovarian [47], cervical [48], lung, and head and neck tumours [49]. Accumulating evidence also indicates that the BRCA genes are somatically inactivated in a percentage of sporadic breast tumours mainly through epigenetic silencing by promoter hypermethylation [50] and/or loss of heterozygosity [51, 52]. This wider involvement of the FA/BRCA pathway in sporadic cancers makes these genes particularly interesting targets for the design of genotype-specific drugs and novel therapeutic options.

As FA-pathway-deficient cells are hypersensitive to DNA interstrand-crosslinking (ICL) agents, patients developing tumours that harbour defects in the FA/BRCA genes could particularly benefit from anticancer treatments based on ICL compounds. Of special interest is not only the identification of new ICL agents but also of compounds conferring sensitivity by mechanisms other than the currently known ICL drugs. These compounds used in combination with ICL drugs might render synergistic effects, allowing for increased effectiveness, dosage reduction and avoidance of side effects [53]. A recent report exploring this avenue has shown by using high-throughput siRNA screening that abrogation of ATM function in FA-pathway-deficient cells results in DNA breakage, cell cycle arrest and apoptotic cell death, cancer cell lines deficient for FA genes being more sensitive to inhibition of ATM than isogenic corrected cell lines [54]. ATM inhibitors could therefore have a role in the targeted treatment of FA-pathway-deficient tumours when used in combination with ICL drugs. In a different work also aiming to identify novel candidate compounds, the growth of *FANCC* and *FANCG* defective human cancer cells was assessed upon treatment with 880 active drugs and 40,000 different agents. By using this high-throughput approach, several potentially interesting candidates that produced hypersensitivity and warrant further investigation were identified [55].

The design of clinical trials represents an important challenge in order to properly evaluate the usefulness of any known or novel compound. However, validation and thorough characterisation of the hypersensitivity of FA-pathway-deficient cells to these agents in suitable preclinical models is necessary before clinical trials can be implemented. Limitations and pitfalls of the existing FA pathway gene defect models have been discussed in detail elsewhere [53]. Briefly, the limitations and artefacts the different models are prone to mainly relate to the type of cells used –human or from other species, normal or malignant, naturally selected for a FA gene defect (i.e., from FA patients) or artificially engineered– or to whether the drug sensitivity is tested *in vitro* or *in vivo* through the xenografting of cell lines in mice [56]. However, despite the variety of models evaluated and the different effects reported, the hypersensitivity of FA-pathway-deficient cells to ICL agents remains a consistent feature, which strengthens the notion that this targeted strategy might indeed translate into clinical success.

Regarding the treatment of *BRCA1*- and *BRCA2*-deficient tumours, a very promising therapeutic strategy has recently emerged. Inhibition of poly (ADP-ribose) polymerase (PARP) sensitises tumour cells to cytotoxic therapy [57] and it has been shown that cells deficient in the BRCA proteins seem to be particularly sensitive to treatment with PARP inhibitors [58, 59]. PARP is a nuclear enzyme that signals DNA damage by adding ADP-ribose units to DNA and histones and participates in the activation of various enzymes involved in different DNA-repair mechanisms, in particular the base excision repair that is key in the repair

of DNA single-strand breaks (SSBs) [60]. PARP inhibition would cause accumulation of such breaks, subsequently resulting in stalled replication forks and accumulation of double-strand breaks (DSBs) [61]. In *BRCA1*- and *BRCA2*-deficient cells where DSB repair by homologue recombination is severely impaired, participation of error-prone alternative mechanisms would lead to complex chromatid rearrangements and eventually to cell cycle permanent arrest or apoptosis, explaining the high sensitivity of these cells to PARP inhibitors [59]. PARP inhibitors are now under evaluation in clinical trials used as single agents or in combination with cytotoxic drugs [62]. Issues such as the selection of the appropriate dose and schedule for the PARP inhibitor (and the other drug if used in combination) will have to be addressed in current and future clinical trials as well as the likeliness of the PARP inhibitor increasing not only efficacy of the other agent but also its toxicity.

The success of effective targeted therapy in tumours arising from FA-BRCA-pathway-deficient cells not only comprises the search for novel compounds conferring sensitivity and the arrangement of suitable clinical trials. Another important challenge ahead is the rapid identification of the subset of patients who will benefit from this tailored therapy [53, 63]. Rapid diagnostic assays that allow detection of FA-BRCA pathway gene defects in tumours will have to be implemented. In the particular scenario of tumours developed by *BRCA1/2* mutation carriers, the identification of a hereditary pattern and quick screening for mutations in the BRCA genes at the time of diagnosis will become decisive. We and others have explored the use of comprehensive immunohistochemical marker panels and high-throughput technologies such as gene expression and array-CGH profiling in order to characterise the different breast tumour subtypes that seem to represent distinct biological entities, both in familial and sporadic breast cancer. The results indicate that about 15% of familial cases not due to mutations in the *BRCA1* or *BRCA2* genes and sporadic cases present a basal phenotype (the same shown by *BRCA1* tumours) and somatic inactivation of both *BRCA1* alleles [64–67]. Therefore, they represent candidate tumours for this type of therapy. More work needs to be done in order to clarify the set of features reflecting the specific consequences of BRCA gene inactivation. However, it is likely that these approaches will gain clinical relevance in the future as tools that might help in the identification and management of suitable patients for treatment regimens designed for BRCA-deficient tumours.

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