The Role of BRCA2 Mutation Status as Diagnostic, Predictive, and Prognosis Biomarker for Pancreatic Cancer

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Pancreatic cancer is one of the deadliest cancers worldwide, and life expectancy after diagnosis is often short. Most pancreatic tumours appear sporadically and have been highly related to habits such as cigarette smoking, high alcohol intake, high carbohydrate, and sugar consumption. Other observational studies have suggested the association between pancreatic cancer and exposure to arsenic, lead, or cadmium. Aside from these factors, chronic pancreatitis and diabetes have also come to be considered as risk factors for these kinds of tumours. Studies have found that 10% of pancreatic cancer cases arise from an inherited syndrome related to some genetic alterations. One of these alterations includes mutation in $BRCA2$ gene. $BRCA2$ mutations impair DNA damage response and homologous recombination by direct regulation of RAD51. In light of these findings that link genetic factors to tumour development, DNA damage agents have been proposed as target therapies for pancreatic cancer patients carrying $BRCA2$ mutations. Some of these drugs include platinum-based agents and PARP inhibitors. However, the acquired resistance to PARP inhibitors has created a need for new chemotherapeutic strategies to target $BRCA2$.

The present systematic review collects and analyses the role of $BRCA2$ alterations to be used in early diagnosis of an inherited syndrome associated with familiar cancer and as a prognostic and predictive biomarker for the management of pancreatic cancer patients.

1. Introduction

In 1994, $BRCA2$ (breast cancer gene 2) was located in chromosome 13q12-13 by the group led by Wooster et al. [1]. Transmission of this gene follows an autosomal dominant pattern with incomplete penetrance [2]. Soon thereafter, $BRCA2$ was reported as a tumour suppressor gene based on evidence of loss of heterozygosity in 7 out of 8 familial breast cancers [3]. Subsequently, $BRCA2$ was associated with high-risk breast and ovarian cancer with a large component of inheritability [4–7], although the risk for ovarian cancer due to $BRCA2$ is much lower than the risk associated with $BRCA1$ [8].

Only one year after this gene was discovered, the association between $BRCA2$ and pancreatic cancer was assessed by Schutte et al. [9]. It was found that pancreatic cancer appeared in some individuals with a history of familial breast cancer associated with $BRCA2$ alterations [10]; thus, it was estimated that 10% of cases of pancreatic cancer have an underlying inherited component [11, 12].

Worldwide pancreatic cancer incidence has increased from 185,000 in the 1980s [13] to 227,000 cases per year in 2014 [14]. In 2007, the highest incidence of pancreatic cancer was in the Baltic countries and central and eastern Europe. In northern European countries and the UK, this cancer has risen over most recent years and is rising in countries of southern, central, and eastern Europe [15]. It is hypothesised that this increase could be associated with increased consumption of high-sugar or carbohydrate-rich foods [16] or simply reflects the ageing of the population in recent decades.

Nowadays, the primary acquired risk factors for pancreatic cancer are cigarette smoking (HR = 1.74), high alcohol consumption (HR = 1.1–1.5), obesity (body mass index > 30; HR = 1.2–1.5), and some infectious diseases that include Helicobacter pylori (HR = 1.5), Hepatitis B virus, or Human Immunodeficiency virus [17–19]. Interestingly, other studies suggested that heavy consumption of cooking and table salt appeared to be significantly associated with pancreatic cancer.
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Figure 1: DNA damage response model performed by BRCA2 and RAD51. Dephosphorylation of BRCA2 at Ser3291 enables RAD51 binding to BRCA2 in BRC repeats and the subsequent binding to double-strand DNA. RAD51 oligomers bind to single-strand DNA at the site of damage that enables its repairs.

$(P = 0.009$ and $P = 0.0001$, resp.), and a similar correlation was found with smoked food $(P < 0.01)$ [20].

Interestingly, observational studies link pancreatic cancer incidence to cadmium, arsenic, and lead exposure [21]. The countries with the highest levels of arsenic (more than $10 \mu g/L$, values recommended by the World Health Organization [22]) are those with highest incidence of pancreatic cancer. These countries include Baltic countries (especially Finland) and central and eastern European countries such as Austria, Czech Republic, Slovakia, and Hungary [23].

Pancreatic cancer has long been related to family history of pancreatic cancer $(HR = 2.20, 95\%, CI = 1.16–4.19)$ and melanoma $(HR = 1.74, 95\%, CI = 1.03–2.95)$, upon breast, ovarian, lung, gastrointestinal, or prostate cancer [24]. In addition, diabetes has also been associated with pancreatic cancer $(HR = 1.4–2.2)$ [25].

Surgical resection is currently the best option so far to improve survival [26]. Mean life expectancy for pancreatic cancer is 1.4 years reaching 3.5 years for surgically resected patients versus 0.8 years for nonoperated patients $(P < 0.001)$ [27]. However, cancers of the pancreas are usually asymptomatic, and the disease only becomes apparent after the tumour invades surrounding tissues or metastasises to distant organs [28]. As a result, there is a pressing need to find new approaches and strategies; of these, targeted therapies hold particular promise, and $BRCA2$ is one such therapy that has great potential. $BRCA2$ regulates sister chromatid cohesion and/or alignment [29] and plays a key role in response to DNA damage by direct regulation of RAD51 recombination (Figure 1).

2. $BRCA2$ in DNA Damage Response

The first attempt to associate $BRCA2$ with DNA damage response was as a cofactor associated with human RAD51-dependent DNA repair of double-strand breaks through 8 evolutionarily conserved BRC motifs encoded in exon II of $BRCA2$ (Figure 1) [30]. The milestone of DNA strand exchange is RAD51 protein which is closely related to other tumour suppressor genes such as $TP53$, $ATM$, $BRCA1$, $BLM$, and $FANCD2$. Preclinical studies showed that $BRCA2$ disruptions sensitize mice embryos to ionising radiation [30], which was previously observed in $RAD51$ knockout mouse embryos [31]. Furthermore, mice carrying truncations on $BRCA2$ loci were one-third smaller than their wild-type littermates and had improper tissue differentiation, sterility, and a shorter overall survival [32,33].

On the other hand, $BRCA2$ is essential for repair of double-strand breaks by homologous recombination [34]. $BRCA2$ alterations led to elevated $P53$ and $P21$ expression, spontaneous accumulation of chromosomal abnormalities, and aberrant chromatid exchanges, which suggests its role in pancreatic tumorigenesis (Figure 2). The aforementioned properties make $BRCA2$ a crucial factor to maintain cell homeostasis.

3. $BRCA2$ as a Prognostic Biomarker of Pancreatic Cancer

$BRCA2$ inactivation is due mainly to genomic mutations. The most common mutations of $BRCA2$ found in pancreatic cancer patients are 6174delT frameshift mutation, 6158insT mutation, splice site mutation 16-2A $>$ G, and the splice site mutation 15-1G $>$ A [35,36]. Another variant located in the 3’-untranslated region is significantly associated with lower expression of $brca2$ RNA and, consequently, with sporadic pancreatic cancer $(HR = 1.3; P < 0.0001)$ [37]. $BRCA2$ inactivation has been reported to be a late event in sporadic pancreatic tumorigenesis [38] preceded by $KRAS$ mutation.
TREX1 were statistically significantly associated with overall MSH2, MSH3, MSH6, PMS2, PMS2L3, RECQL, TP73, and shortening and progression of cancer [46]. Several MMR genes microsatellites are found in oncogenes associated with initiation and progression, which become unstable. Alterations in (MMR) family genes [45]. MMR genes allow continuation.

ATM, CDKN2, PALB2, PRSS1, STK11, or associated with mutations not only in directly related to patient outcome. PanIN: pancreatic intraepithelial neoplasia; CIN: chromosome instability; LOH: loss of heterozygosity.

(G12D) or loss of TP53 [39, 40]. Overall, BRCA2 could be used to determine patient prognosis. Ashkenazi Jews have been one of the most closely studied ethnic groups concerning the significance of BRCA2 mutations and family involved pancreatic cancer. Struwing et al. found that >90% of Ashkenazi patients that carried BRCA2 mutations detected in blood sample showed an association with increased risk of pancreatic cancer [41]. The 6174delT mutation of BRCA2 was determined to be present in 1% (CI = 0.6–1.5) of 1.255 Jewish individuals [42]. Another study performed with 26 European families reported that 19% (CI = 7% to 39%) of the families with first-degree relatives with pancreatic cancer had either a mutation or a splice variant of BRCA2 [43]. Murphy et al. reported 17% of BRCA2 mutations in 31 samples from pancreatic cancer patients with at least two first-degree relatives affected by pancreatic cancer [35]. One study found 6% (10 of 180 families) with BRCA2 mutation and moderate or high-risk pancreatic cancer predisposition and 6% (8 of 146) of families that presented two or more first-degree relatives affected with pancreatic cancer [44].

This kind of studies performed with high-risk pancreatic cancer families provides a true enlightenment of BRCA2-associated pancreatic cancer; however, BRCA2 has not been directly related to patient outcome. Pancreatic cancers with a high familial component are associated with mutations not only in BRCA2 but also in ATM, CDKN2, PALB2, PRSS1, STK11, or mismatch repair (MMR) family genes [45]. MMR genes allow continuous point mutations in repeats regions of DNA known as microsatellites that become unstable. Alterations in microsatellites are found in oncogenes associated with initiation and progression of cancer [46]. Several MMR genes are involved in the acquisition of aggressive phenotype of cancer [47]. For example, alterations on EXO1, MLH1, MSH2, MSH3, MSH6, PMS2, PMS2L3, RECQL, TP73, and TREX1 were statistically significantly associated with overall survival of pancreatic cancer patients [48]. Nevertheless, the predisposition to pancreatic cancer by MMR family genes is due mainly to mutations in MLH1 and MSH2 and it is estimated to be <5% [49].

4. BRCA2 as Predictive Biomarker in Pancreatic Cancer

BRCA2 mutations impair DNA repair; thus, they are considered biomarkers of genomic instability and DNA damage repair deficiency. Therefore, BRCA2 mutations could be used as predictive biomarkers of response to some DNA damage agents. Some of these compounds include platinum-based agents and PARP inhibitors. They are considered targeted therapies indicated for BRCA2-positive tumours according to some good results achieved in clinical trials [50]. Platinum-based drugs are, despite their toxicity, one of the gold-standard chemotherapies administered to pancreatic cancer patients. Cisplatin, carboplatin, and oxaliplatin are some of the mostly used in clinical practice and allow cross-linking and forming DNA adducts which trigger apoptosis cascade [51].

Oliver et al. presented a cohort of pancreatic cancer patients; of them, those with family history of breast, ovarian, or pancreatic cancers showed significantly increased survival after platinum-based chemotherapy compared to other patients without family history (22.9 versus 6.3 months, P < 0.01) [52]. A case report of a 60-year-old pancreatic adenocarcinoma patient carrying the BRCA2 mutation, 1153insertionT, presented recurrence after gemcitabine treatment but showed a complete response after cisplatin and gemcitabine as second-line therapy [53]. Subsequently, another study with pancreatic cancer patients with positive BRCA1/2 mutations showed improved outcome after treatment with platinum-based chemotherapy. Here, patients with locally advanced disease were pathologically downstaged and those with metastatic disease had significant increase in their progression-free survival [54]. Golan et al. reported that stage III/IV patients treated with platinum-based chemotherapy carrying BRCA1/2 mutations had improved overall survival compared to those patients treated with other drugs (22 versus 9 months, resp.; P = 0.039) [55]. One study reported that 5 out of 8 patients with pancreatic ductal adenocarcinomas that were treated with platinum-based chemotherapy presented BRCA2 mutation. Of these 5 patients, 2 had complete radiological response and 2 had partial responses to platinum treatment [56]. All the above-mentioned studies suggest that BRCA2 mutations predict not only platinum response but also better outcome and longer survival for pancreatic cancer patients with advanced disease. Poly(ADP-ribose) polymerase inhibitors (PARPi) prevent the repair of double-strand DNA breaks, homologous recombination, and replication repair performed by the PARP family of proteins [57]. A preclinical study with CAPAN-1 cell line has suggested that 6174delT mutation of BRCA2 is highly sensitive to PARPi [58]. However, another study also performed with pancreatic cancer cell lines reported how a PARPi increases sensitivity to chemoradiotherapy independently of BRCA2 mutation status [59].
Table 1: Clinical trials for BRCA2 mutated pancreatic cancer patients.

| Clinical trial   | Phase                  | Study type         | Drugs                                      | Sponsor                        | Inclusion criteria                                                                 |
|------------------|------------------------|--------------------|--------------------------------------------|-------------------------------|-----------------------------------------------------------------------------------|
| NCT02309632      | Screening              | Nonrandomized      | Screening of high-risk individuals        | University of Arkansas        | Peutz-Jegher's Syndrome, BRCA1 mutation carrier, BRCA2 mutation carrier, Ataxia-telangiectasia, Familial atypical malignant melanoma syndrome, Colorectal neoplasms, hereditary nonpolyposis, Hereditary pancreatitis |
| NCT02000089      | Prospective observational | Cohort           | Human synthetic secretin                  | Johns Hopkins University      | Pancreas cancer, Peutz-Jeghers Syndrome, Gene mutation, Germline mutation carrier, Lynch Syndrome |
| NCT02775461      | Prospective observational | Cohort           | —                                            | Icahn School of Medicine at Mount Sinai | Pancreas cancer, Pancreatitis, Chronic pancreatitis, Pancreatic cyst, Family history of pancreas cancer, Genetic mutations |
| NCT01585805      | Phase II               | Randomized         | Gemcitabine, cisplatin with or without veliparib or veliparib alone | National Cancer Institute     | BRCA1 mutation carrier, BRCA2 mutation carrier, Metastatic pancreatic adenocarcinoma, Pancreatic adenocarcinoma, Recurrent pancreatic carcinoma, Stage III pancreatic cancer, Stage IV pancreatic cancer |
| NCT01102569      | Prospective observational | Cohort           | —                                            | Columbia University           | BRCA1 mutation carrier, BRCA2 mutation carrier |
| NCT00438906      | Prospective observational | Cohort           | Human synthetic secretin                  | Johns Hopkins University      | Pancreatic neoplasm, Peutz-Jeghers Syndrome |
| NCT01233505      | Phase I                | Interventional     | Veliparib, oxaliplatin, capecitabine       | National Cancer Institute     | Advanced solid tumors, BRCA1 mutation carrier, BRCA2 mutation carrier |
| NCT02703545      | Prospective observational | Cohort           | —                                            | Johns Hopkins University      | Peutz-Jeghers Syndrome, Familial pancreas cancer, BRCA1 mutation carrier, BRCA2 mutation carrier, Hereditary pancreatitis |
| NCT00714701      | Prospective observational | Cohort           | —                                            | Sidney Kimmel Comprehensive Cancer Center | Early pancreatic neoplasia, Familial pancreatic neoplasia |
| NCT00892736      | Phase I                | Interventional     | Veliparib                                   | National Cancer Institute     | Advanced solid tumors, BRCA1 mutation carrier, BRCA2 mutation carrier, Estrogen receptor negative, HER2/Neu negative |
A different drug popularly used in pancreatic cancer treatment is gemcitabine and is able to induce DNA damage response and PARP degradation [60]. Gemcitabine in combination with PARPi showed promising antitumor activity compared to PBS, gemcitabine, or PARPi alone, in in vivo models of pancreatic cancer [61].

In clinical studies, BRCA2-positive status has been associated with better response to PARPi alone or in combination with other drugs. In one study, 3 out of 4 patients with a known BRCA1 or BRCA2 mutation showed partial response after receiving PARPi alone or in combination with platinum-based chemotherapy [62]. In a phase I/II trial of PARPi in combination with 5FU and oxaliplatin that included 2 patients with BRCA2 mutation, one showed a partial response and the other achieved complete response [63]. Another phase IB trial of PARPi in combination with gemcitabine and platinum-based chemotherapy reported that BRCA-mutated patients achieved partial response in 56% and stable disease in 44% of cases. However, 62% of BRCA wild-type patients remained with stable disease and 25% with progression [64].

To date, personalised therapies in pancreatic cancer could improve patient survival if assisted by breakthrough techniques used in molecular diagnosis. Deep sequencing currently offers a high-throughput method of dissecting the underlying mechanisms of tumorigenicity, leading to new strategies for personalised medicine. However, pancreatic cancer genotype is highly heterogeneous, and this heterogeneity involves its therapeutic ineffectiveness [65]. The IMPaCT clinical trial was set out to improve patient survival using deep sequencing to guide treatment decisions. In the study, patients carrying BRCA2 mutations were eligible to receive targeted treatment based on 5FU and mitomycin versus gemcitabine alone [66]. Nevertheless, no consistent conclusions arose from this trial due to the low number of patients recruited with BRCA2 mutations [67].

Nevertheless, patients could present acquired resistance to platinum-based chemotherapy by accumulation of secondary genomic alterations, such as BCR-ABL point mutations, in which case the BRCA2 mutation effect is bypassed [68].

Table 1 summarises ongoing or recently completed clinical trials recruiting BRCA2 mutated pancreatic cancer patients.

5. Conclusions

Pancreatic cancer is one of the most deadly cancers worldwide, and despite new methods of early diagnosis, surgery, and drug discovery, tumour cells tend to scatter and metastasise to vital organs, thereby reducing survival significantly. It is also highly resistant to treatments and responds poorly to chemoradiotherapy; indeed, chemoradiotherapy is used in most of cases as a palliative therapy. Therefore, patients are encouraged to participate in clinical trials regardless of disease stage.

Some studies attribute the increasing incidence of sporadic pancreatic cancer to the ageing of the population. However, several studies have reported different factors associated with this neoplasm. Obesity, cigarette smoking, high alcohol intake, and chronic pancreatitis are the most relevant factors [69].

On the other hand, it is estimated that 10% of pancreatic cancer cases are due to an inherited syndrome [11, 12] caused by mutations in the BRCA1 or BRCA2 genes [10]. Most of the clinical studies that relate pancreatic cancer to BRCA2 mutations have been performed on Ashkenazim. Although this fact limits the findings’ applicability to other populations, there is nonetheless great potential in the study of the heritability of BRCA2 mutation and pancreatic cancer incidence [41, 42].

Several preclinical and clinical studies have suggested the potential use of BRCA2 mutations as biomarkers for DNA damage agents’ response like platinum-based chemotherapy and PARPi. Clinical trials have evaluated BRCA2 as a predictive biomarker for use in platinum-based therapies but they were mainly retrospective and with a scarce cohort of patients. Thus, further multicenter prospective studies using larger cohorts are required to investigate multitarget therapies and their potential to minimize resistance to therapy.

Competing Interests

The authors declare no conflict of interests.

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References

[1] R. Wooster, S. L. Neuhausen, J. Mangion et al., “Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13,” Science, vol. 265, no. 5181, pp. 2088–2090, 1994.
[2] D. L. Thull and V. G. Vogel, “Recognition and management of hereditary breast cancer syndromes,” Oncologist, vol. 9, no. 1, pp. 13–24, 2004.
F. J. Couch, M. R. Johnson, K. G. Rabe et al., “The prevalence

N. Habbe, P. Langer, M. Sina-Frey, and D. K. Bartsch, “Familial

C. Oddoux, J. P. Struwing, C. M. Clayton et al., “The carrier

B. Tran, G. Zogopoulos, A. Borgida, S. Holter, S. Gallinger,

G. Luo, Y. Lu, K. Jin et al., “Pancreatic cancer: BRCA2 mutation

J. Jiricny, “The multifaceted mismatch-repair system,”

S. A. Hahn, B. Greenhalf, I. Ellis et al., “BRCA2 germline

M. Rowley, A. Ohashi, G. Mondal et al., “Inactivation of Brca2

L. Huan, C. Wu, D. Yue et al., “Identification of common

M. Goggins, R. H. Hruban, and S. E. Kern, “BRCA2 is

M. R. Zhao, P. Langer, M. Sina-Frey, and D. K. Bartsch, “Familial

T. Golan, Z. S. Kanji, R. Epelbaum et al., “Overall survival and
clinical characteristics of pancreatic cancer in BRCA mutation
carriers,” British Journal of Cancer, vol. 111, no. 6, pp. 1132–1138,
2014.

N. Waddell, M. Pajic, A.-M. Patch et al., “Whole genomes
redefine the mutational landscape of pancreatic cancer,” Nature,
vol. 518, no. 7540, pp. 495–501, 2015.

J. M. Lee, J. A. Ledermann, and E. C. Kohn, “PARP inhibitors
for BRCA1/2 mutation-associated and BRCA-like malignan-
cies,” Annals of Oncology, vol. 25, no. 1, Article ID mdt384, pp. 32–40, 2014.

N. McCabe, C. J. Lord, A. N. J. Tutt, N. M. B. Martin, G. C.
M. Smith, and A. Ashworth, “BRCA2-deficient CAPAN-1 cells
are extremely sensitive to the inhibition of poly (ADP-Ribose)
polymerase: an issue of potency,” Cancer Biology and Therapy,
vol. 4, no. 9, pp. 934–936, 2005.

L. Porcelli, A. E. Quattrole, P. Mantuano et al., “Optimize
radiochemotherapy in pancreatic cancer: PARP inhibitors a
new therapeutic opportunity,” Molecular Oncology, vol. 7, no. 3,
pp. 308–322, 2013.

Y. Wang, Y. Kuramitsu, K. Tokuda et al., “Gemcitabine induces
poly (ADP-ribose) polymerase-1 (PARP-1) degradation through
autophagy in pancreatic cancer,” PLoS ONE, vol. 9, no. 10,
Article ID e109076, 2014.

D. A. Jacob, M. Bahra, J. M. Langreh et al., “Combination
therapy of poly (ADP-ribose) polymerase inhibitor 3-
aminobenzamide and gemcitabine shows strong antitumor
activity in pancreatic cancer cells,” Journal of Gastroenterology
and Hepatology, vol. 22, no. 5, pp. 738–748, 2007.

M. A. Lowery, D. P. Kelsen, Z. K. Stadler et al., “An emerging
entity: pancreatic adenocarcinoma associated with a known
brca mutation: clinical descriptors, treatment implications, and
future directions,” Oncologist, vol. 16, no. 10, pp. 1397–1402, 2011.

M. J. Pishvaian, H. Wang, T. Zhuang et al., “A phase II/II study
of ABT-888 in combination with 5-fluouracil (5-FU) and
oxaliplatin (Ox) in patients with metastatic pancreatic cancer
(MPC),” Journal of Clinical Oncology, vol. 31, supplement 4,
abstract 147, 2013.

E. M. O’Reilly, M. A. Lowery, M. F. Segal et al., “Phase IB trial
of cisplatin (C), gemcitabine (G), and veliparib (V) in patients
with known or potential BRCA or PALB2-mutated pancreatic
cancer (PC),” Journal of Clinical Oncology, vol. 30, no. 5,
supplement, abstract 4023, 2014.

A. V. Biankin, N. Waddell, K. S. Kassahn et al., “Pancreatic
cancer genomes reveal aberrations in axon guidance pathway
genes,” Nature, vol. 491, no. 7424, pp. 399–405, 2012.

D. D. Von Hoff, T. I. Ervin, F. P. Arena et al., “Results of a
randomized phase III trial (IMPACC) of weekly nab-paclitaxel
plus gemcitabine versus gemcitabine alone for patients with
metastatic adenocarcinoma of the pancreas with PET and
CA19-9 correlates,” in Proceedings of the ASCO Annual Meeting,
abstract 4005, 2013.

L. A. Chantrill, A. M. Nagrial, C. Watson et al., “Precision
medicine for advanced pancreas cancer: the individualized
molecular pancreatic cancer therapy (IMPACCT) Trial,” Clinical
Cancer Research, vol. 21, no. 9, pp. 2029–2037, 2015.

W. Sakai, E. M. Swisher, B. Y. Karlan et al., “Secondary mutations
as a mechanism of cisplatin resistance in BRCA2-mutated
cancers,” Nature, vol. 451, no. 7182, pp. 1116–1120, 2008.
[69] U. Nöthlings, L. R. Wilkens, S. P. Murphy, J. H. Hankin, B. E. Henderson, and L. N. Kolonel, “Meat and fat intake as risk factors for pancreatic cancer: the multiethnic cohort study,” *Journal of the National Cancer Institute*, vol. 97, no. 19, pp. 1458–1465, 2005.