FULL PAPER

BJC

British Journal of Cancer (2015) 113, 425–432 | doi: 10.1038/bjc.2015.220

Keywords: BRCA1; BRCA2; BRCAness; pancreatic cancer; patient-derived pancreatic cancer xenograft; cisplatin; gemcitabine

BRCA1 and BRCA2 mutations sensitize to chemotherapy in patient-derived pancreatic cancer xenografts

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Background: Germline mutations of the BRCA tumour suppressors have been associated with increased risk of pancreatic cancer. Clinical evidence suggests that these patients may be more sensitive to treatment with cisplatin. As the frequency of germline BRCA mutations is low, definitive experimental data to support the clinical observations are still missing.

Methods: We tested gemcitabine and cisplatin sensitivity of four BRCA1 and BRCA2 mutant and three BRCA1 and BRCA2 wild-type (WT) patient-derived pancreatic cancer xenografts.

Results: We observed treatment sensitivity to gemcitabine and cisplatin in the BRCA WT and mutant models. The BRCA1 and BRCA2 mutant xenografts were significantly more sensitive to cisplatin although these models also showed sensitivity to gemcitabine. The BRCA1 and BRCA2 WT models showed sensitivity to gemcitabine but not cisplatin. Treatment sensitivity in the xenograft models closely resembled treatment response in the corresponding patients.

Discussion: We have characterised a panel of xenografts derived from pancreatic cancer patients carrying germline BRCA mutations, and shown that their genetic features resemble the patient donor. Our results support further clinical testing of treatment regimens combining gemcitabine and platinum drugs in this patient population, as well as preclinical research aiming to identify mechanisms of cisplatin resistance in BRCA mutant pancreatic cancers.

It has been estimated that up to 10% of pancreatic cancers have a hereditary component with autosomal dominant transmission of mutations in tumour suppressors as the prevalent genetic basis for increased risk (Ghadirian *et al*, 1991; Fernandez *et al*, 1994; Klein *et al*, 2001; Bartsch *et al*, 2004; Grant *et al*, 2014). Although germline mutations in several known cancer susceptibility genes have been implicated in increased risk of pancreatic cancer (Hruban *et al*, 2010), mutations in the breast cancer early onset (BRCA1 & BRCA2) tumour suppressor genes are currently the best characterised and appear to be to be responsible for $\sim$15% of familial cases (Couch *et al*, 2007). Patients with BRCA2 mutation have been reported to have an overall 3.5-fold risk of developing pancreatic cancer compared with the general population (The Breast Cancer Linkage Consortium, 1999).

The BRCA tumour suppressors are involved in the repair of DNA double-strand breaks (DSB) and DNA cross-linking damage induced by DNA-damaging agents through the homologous recombination (HR) pathway. The BRCA1 and BRCA2 are

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Revised 3 May 2015; accepted 15 May 2015; published online 16 July 2015

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localised to the nucleus in response to DNA damage leading to the formation of RAD51 foci and subsequent DNA damage repair (Venkitaraman, 2001; Gudmundsdottir and Ashworth, 2006). The lack of RAD51 foci implies defects in the HR pathways (Gudmundsdottir and Ashworth, 2006). In addition to its function in HR, BRCA2 function is essential for non-HR repair (Venkitaraman, 2001; Gudmundsdottir and Ashworth, 2006).

Clinical data in ovarian cancer show that patients with BRCA1 and BRCA2 mutations show higher response rates to treatment with cisplatin and other DNA-damaging agents resulting in improved outcome (Dann *et al*, 2012; Muggia and Safra, 2014). Similar observations have been made in case reports of patients with pancreatic cancer, suggesting that BRCA1 and BRCA2 mutations may sensitise to treatment with platinum drugs (Lowery *et al*, 2011; Sonnenblick *et al*, 2011). This is supported by preclinical studies using established pancreatic cancer cell lines (van der Heijden *et al*, 2005), and also by a large retrospective analysis of 71 pancreatic cancer patients with germline BRCA1 and BRCA2 mutations, where it was reported that patients with stage 3/4 disease who were treated with a platinum-containing regimen had a median survival of 22 months, compared with 9 months for those who did not receive platinum (Golan *et al*, 2014).

There are important unanswered questions concerning the optimum design of platinum-containing treatment protocols for this group of patients, as well as the role of newer agents such as inhibitors of poly-ADP-ribose polymerase (PARP) that have shown early promise in other cancer patients carrying germline BRCA mutations. Owing to the relatively low frequency of these mutations in pancreatic cancer patients, it is difficult to address these questions efficiently using prospective clinical trials. As primary xenografts derived from pancreatic cancers appear to maintain characteristics of the patient donor (Chang *et al*, 2011; Lohse *et al*, 2014), they offer an alternative, 'near-clinical' approach for the development and testing of treatment protocols for patients with BRCA mutations. However, the extent to which primary xenografts derived from BRCA mutant pancreatic cancers maintain the genetic and phenotypic features of the patient donor remains unclear, and is of particular concern given the potential for genomic instability conferred by BRCA mutations. These questions are addressed in the present paper.

MATERIALS AND METHODS

Patients. Subcutaneous and orthotopic tumours of seven primary xenografts, designated as Ontario Cancer Institute Pancreas (OCIP) 19, 23, 28, 167, 217, 232 and A1, were established from pancreatectomy samples superfluous to patient diagnosis or ascites samples using a protocol approved by the University Health Network Research Ethics Board. Informed consent was obtained from all the participating patients.

Patients OCIP28 (http://www.ncbi.nlm.nih.gov/clinvar/RCV000044800/), OCIP217 (http://www.ncbi.nlm.nih.gov/clinvar/RCV000019244/), OCIP232 and OCIPA1 had clinically relevant, deleterious germline mutations in BRCA1 and BRCA2 (Table 1). The presence of the patient mutation was confirmed in the xenografts by Sanger sequencing.

Primary patient-derived xenografts. Animal experiments were carried out using protocols approved by the University Health Network (UHN) Animal Care Committee under the guidelines of the Canadian Council on Animal Care. Primary xenografts were established from pancreatectomy samples as previously described (Chang *et al*, 2011; Lohse *et al*, 2014). Briefly, tumour fragments were implanted subcutaneously into the flank of 4–5-week-old non-obese diabetic severe combined immune-deficient mice (NOD/SCID). All the models used in this study showed first-generation growth and 100% take rate from the third passage on and can be regrown from cryopreserved tumour fragments. The xenograft models closely resemble the morphology of the patient specimen (Chang *et al*, 2011; Lohse *et al*, 2014) and show stable growth rates over multiple passages. Subcutaneous tumours were measured using callipers, and volume calculated according to the formula width^2 $\times$ length $\times$ 0.5.

Treatments were started when tumour volume reached $\sim$150 mm^3. Animals were treated with 4 mg kg^{-1} cisplatin (Sigma-Aldrich, Oakville, ON, Canada) weekly intraperitoneal or 100 mg kg^{-1} gemcitabine (Accord Healthcare Inc, North Harrow, UK) biweekly intraperitoneal for 30 days. Animals were killed when tumours reached humane end point according to institutional guidelines.

For acute treatments, animals were treated with a single dose of 4 mg kg^{-1} cisplatin and tumours were excised for analysis 24 h after treatment.

Histological analysis. For BRCA1, BRCA2 and RAD51 staining, tumours were excised, fixed and paraffin embedded. Paraffin tissue sections were cut, dried and dewaxed. Following peroxidase block and antigen retrieval, sections were incubated for 1 h at room temperature with antibodies against RAD51 (Santa Cruz, Dallas, TX, USA, sc-8349, 1:100), BRCA1 (Santa Cruz, sc-1021, 1:100) and BRCA2 (Santa Cruz, sc-1818, 1:50). This was followed with AlexaFluor 555 labelled secondary (Invitrogen, Burlington, ON, Canada) for 60 min. DAPI (Sigma-Aldrich, Oakville, ON, Canada) was applied for 5 min. The slides were then air dried and stored at 4 °C. Images were acquired using the Olympus IX81 Spinning Disk Confocal Microscope (Richmond Hill, ON, Canada). Tumours containing nuclei with more than five nuclei per cells were counted as positive (Fraser *et al*, 2011).

To determine the level of tumour hypoxia, mice were injected intraperitoneally with the 2-nitroimidazole hypoxia marker EF5, 30 mg kg^{-1}, 3 h before being killed (Lord *et al*, 1993; Koch, 2002). Tumours were excised and processed as described previously (Chang *et al*, 2011; Lohse *et al*, 2014). Sections were labelled with primary antibodies to α-smooth muscle Actin (α-SMA), (DAKO, Glostrup, Denmark, clone 1A4, 1:400), CD31 (Santa Cruz, sc-1506, 1:1000) overnight. Biotinylated anti-mouse IgG incubations were carried out followed by streptavidin biotin detection system (Signet Pathology System, Deham, MA, USA). For EF5 staining, slides were incubated in biotinylated EF5 antibody (obtained from Dr Cameron Koch, University of Pennsylvania, Philadelphia, PA, USA, 1:250) (Lord *et al*, 1993; Koch, 2002). Sections were scanned at $\times$20 resolution using an Aperio Scanscope XT scanner (Aperio Technologies, Vista, CA, USA). Images were analysed using the Aperio ImageScope software ver. 11.1.2.752, positive pixel count algorithm (PPC). Necrotic areas were excluded from the analysis.

For double-fluorescent staining, paraffin tissue sections were cut, dried and dewaxed. After antigen retrieval and serum block, sections were incubated at room temperature with γH2AX (EMD Millipore, Etobicoke, ON, Canada, clone JBW301, 1:1000) and cleaved caspase-3 (CC3) (Cell Signaling, Danvers, MA, USA, #9661, 1/200) cocktail overnight. Secondary antibodies (goat anti-mouse AlexaFluor 647 and goat anti-rabbit AlexaFluor 555, Invitrogen, 1/100) were applied for 1 h at room temperature. Sections were stained with DAPI for 10 min at room temperature and air dried.

Images of the entire section were obtained using a multilaser scanner (TS4000; Huron Technologies, Waterloo, ON, Canada) at 0.5 μm per pixel. Region of tumour, necrosis, stroma, folds were specified, creating a training rule-set for tissue recognition. Cellular analysis included nucleus identification and separation; objects $<10\,\mu m^2$ were excluded. Individual nuclear mean intensities for γH2AX and CC3 channels were recorded. A threshold was

Table 1. Primary pancreatic cancer resection specimen

Model	Sex	Diagnosis	Grade	Clinical stage[a]	Pathology stage[a]	Surgery	Recurrence	Germline BRCA mutation[b]	Survival (days)
OCIP19	M	Ductal ADC	G2	T3NxM0	T3N1b	Y	Local	WT	562
OCIP23	M	Ductal ADC	G3	T3NxM0	T3N1b	Y	Distant	WT	249
OCIP28	F	Ductal ADC	G1	T4N0M0	T3N0	Y		BRCA2 c.6174delT p.S1982Rfs	2047[c]
OCIP167	M	Ductal ADC	G2	T2NxM0	T3N1b	Y	Distant	WT	1150
OCIP217	F	Ductal ADC	G3	T4N0M1	T2N0	Y	Distant	BRCA1 c.4327C>T, p.R1443X	777
OCIP232	M	Ductal ADC	G2	TxNxM1	T3N1b	Y	Distant	BRCA2 c.3393delC p.L1059X	681
OCIPA1	M	Ductal ADC	G3	T3N1M1	/	N[d]	N	BRCA2 c.1736T>G, p.L579X	355

Abbreviations: F = female; M = male; N = no; WT = wild type; Y = yes.
[a]TNM classification of tumours of the exocrine pancreas: T2, tumour limited to the pancreas, more than 2 cm in greatest dimension; T3, tumour extends directly into any of the following: duodenum, bile duct, peripancreatic tissues; T4, tumour extends directly into any of the following: stomach, spleen, colon, adjacent large vessels; N0, no regional lymph node metastasis; N1a, metastasis in a single regional lymph node; N1b, metastasis in multiple regional lymph nodes.
[b]c, change in nucleotide sequence; p, change in protein sequence.
[c]Patient alive.
[d]Xenograft model derived from ascites.

determined by mean + 5 × s.d. intensity in the γH2AX and CC3 channels. No image processing was carried out before the analysis.

For p53 (Vector Labs, Burlington, ON, Canada, 1:250), p16 (Abcam, Cambridge, MA, USA, 1:100) and Smad4 (Abcam, 1:100) stainings, paraffin tissue sections were cut, dried and dewaxed. Following peroxidase block and antigen retrieval sections were incubated for 1 h at room temperature with primary antibodies. After incubation in biotinylated anti-rabbit IgG (Vector Labs, 1:200) followed by HRP labelling reagent (Signet Pathology System Inc) for 30 min, immunoreactivities were revealed by incubation in Nova Red substrate (Vector Labs) for 5 min and counterstained in Mayer's haematoxylin. Slides were scanned at × 20 resolution using an Aperio Scanscope XT scanner, and the percentage of positive pixels determined using the Aperio Imagescope software (Vs.11.1.2.752, Aperio Technologies).

Statistical analysis. The tumour growth data were analysed using mixed effect modelling which accounts for the correlations among the measurements of the same model. The tumour volume was transformed to the linear scale (power 1/3) to stabilise the variance of the residuals. The survival percentages for the *in vivo* data were calculated using the Kaplan–Meier technique and the curves were tested for significance using the log-rank test. The changes in DNA damage as measured by γH2AX and CC3 were analysed by applying the mixed effect modelling to mitigate the existent correlations between the measurements of the same model. Both markers needed to be log-transformed to obtain residuals distributed normally.

RESULTS

BRCA1 and BRCA2 patient-derived xenografts. For this study, we used a set of four pancreatic cancer xenografts established from patients with known germline mutations in the BRCA 1 and BRCA2 genes (Table 1). The BRCA1 and BRCA2 mutants express truncated proteins with C-terminal deletions (Figure 1A and B). These models were compared with three wild-type (WT) xenografts without germline or somatic mutations in BRCA1 or BRCA2. The control models were selected to match the BRCA mutants in terms of growth rate as indicated by the time elapsed between two passages, magnitude of hypoxia as shown by staining for the hypoxia marker EF5 and stromal content as indicated by αSMA staining (Supplementary Figure 1A–C).

The OCIP217 model expresses a truncated version of BRCA1 that has lost the BRCT domain involved in BRCA1 recruitment to the DNA damage site and part of the ATM-binding domain (Figure 1A). Loss of heterozygosity was demonstrated in the patient-derived xenograft when compared with the patient specimen.

The OCIP28, OCIP232 and OCIPA1 models express truncated versions of BRCA2 of different lengths (Figure 1B). The OCIP28 model expresses a 2002 amino acid (AA) protein that has lost its nuclear localisation sequence (NLS), the oligonucleotide binding domains (OB) as well as part of the RAD51-binding domain. Although the patient tumour that OCIP28 was derived from retained the WT BRCA2 allele, loss of heterozygosity occurred in the patient-derived xenograft. OCIP232 carries a germline mutation that gives rise to a 1059AA version of BRCA2 that has lost most of the RAD51-binding domain. In addition, the OCIP232 xenograft model carries a somatic mutation (c.G8909A, p.W2970X). This mutation results in the expression of a truncated BRCA2 protein, while maintaining the RAD51-binding domain has lost the NLS and OB domains. OCIPA1 expresses the shortest BRCA2 protein with 579AA. The WT allele was retained in the OCIPA1 xenograft.

To evaluate BRCA1 and BRCA2 function, animals were treated with a single dose of cisplatin and BRCA1, BRCA2 and RAD51 expression evaluated 24 h after treatment.

The WT models displayed an increase in both BRCA1 and BRCA2 expression in response to treatment with cisplatin (Supplementary Figure 1D and E) and cytoplasmic and nuclear foci. Similar staining patterns were observed in the mutant xenografts stained for BRCA1 with the exception of OCIPA1, which did not show any BRCA1 staining. The OCIP28 tumours stained for BRCA2 showed no increase in staining in response to cisplatin treatment, while staining levels were similar to WT controls. The OCIP217, 232 and A1 models displayed a mostly

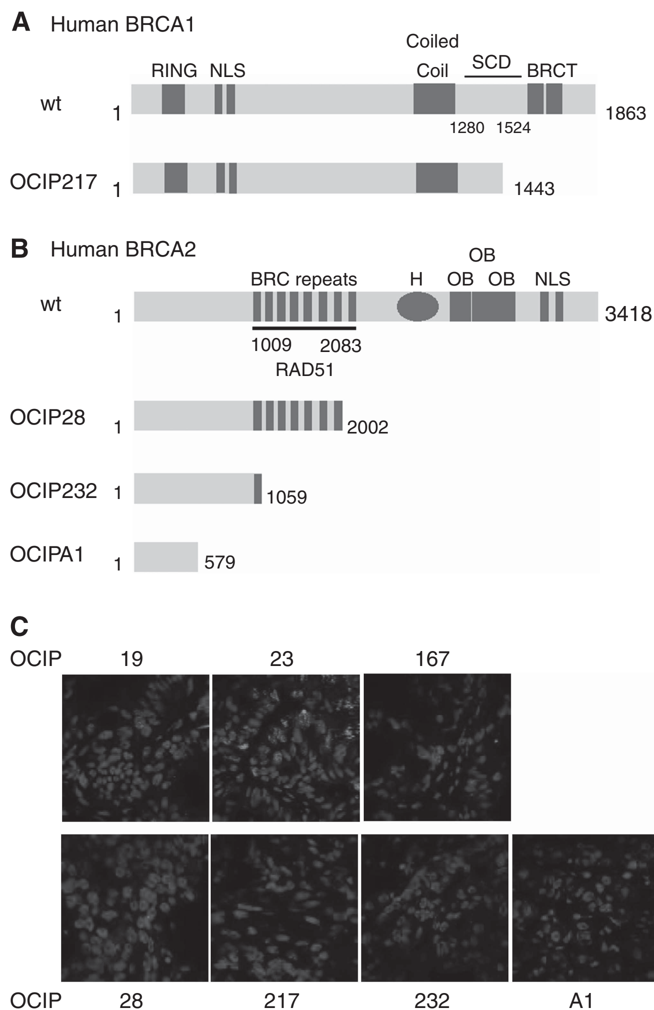

Figure 1. BRCA1 and BRCA2 mutants. (**A**) The BRCA1 amino terminus contains a RING domain and the nuclear localisation sequence (NLS). The carboxyl terminus contains the coiled coil domain that facilitates BRCA2 binding, a SQ/TQ cluster domain (SCD) that contains 10 possible ATM phosphorylation sites and a BRCT domain, which is associated with BRCA1 recruitment to the DNA damage site. The mutation found in OCIP217 leads to the expression of a truncated BRCA1 protein that has lost the BRCT domain and parts of the ATM SCD. (**B**) BRCA2 contains eight BRC repeats between amino acids (AA) 1009 and 2083 that binds RAD51. The carboxyl terminus contains helical domain (H), three oligonucleotide binding (OB) domains, which facilitates binding to both single-stranded and double-stranded DNA, and an NLS. All BRCA2 mutants express truncated proteins that have lost the carboxyl terminus. While OCIP28 maintains seven BRC repeats, OCIP232 and OCIPA1 have lost the RAD51-binding domains. Cellular localisation of (**C**) RAD51(red) in sections of xenograft tumours treated with vehicle or cisplatin. DAPI (blue) was used to visualise the cell nucleus.

diffuse cytoplasmic staining pattern (Supplementary Figure 1D and E).

Consistent with a functional DNA double-strand break (DSB) repair system, all the WT models displayed RAD51 foci in response to treatment with cisplatin (Figure 1C). No RAD51 foci were observed in the BRCA1 and BRCA2 mutant xenografts in response to treatment with cisplatin (Figure 1C).

The WT and BRCA mutant xenografts were characterised for mutations in Kras, p53, p16 and Smad4 (Supplementary Table 1). All the models show mutation in Kras codon 12. Three of the seven models (OCIP23, 232, A1) show mutations in p53. Loss of p16 was observed in OCIP28, 167, 232 and A1. With the exception of OCIP167 and OCIPA1, Smad4 was expressed in all the models.

BRCA1 and BRCA2 mutations sensitise to treatment. To test sensitivity to gemcitabine (Gem) and cisplatin (Cis) *in vivo*, tumour-bearing mice were treated with 4 weeks of gemcitabine, cisplatin or vehicle.

The BRCA WT xenograft models OCIP19, OCIP23 and OCIP167 show significantly reduced tumour growth ($P<0.0001$) in response to treatment with gemcitabine compared with vehicle or cisplatin-treated tumours (Figure 2A). Although treatment with cisplatin significantly reduced tumour growth in OCIP167 ($P<0.0001$), the effect is less pronounced. OCIP19 ($P=0.016$) and OCIP23 ($P<0.0001$) showed little response to cisplatin treatment. Treatment with gemcitabine consistently resulted in a significant increase in survival in OCIP19 ($P=0.029$) and OCIP167 ($P=0.0011$) while treatment with cisplatin only led to a mild increase in survival ($P=0.013$ and 0.0011, respectively for OCIP19 and OCIP167). Neither treatment increased survival in OCIP23 (Figure 2B).

In contrast, both gemcitabine and cisplatin significantly reduced tumour growth in all the BRCA mutant xenograft models ($P<0.0001$). Both treatments are significantly more potent in BRCA mutant xenografts than BRCA WT models ($P<0.0001$). In the BRCA2 mutant OCIP28 and the BRCA1 mutant OCIP217, treatment with either drug resulted in complete regression of the tumours (Figure 2A), although regrowth occurred in some of the treated mice. Both gemcitabine and cisplatin significantly prolonged survival compared with untreated control ($P<0.0001$), with some treated mice dying of old age without any palpable tumours (Figure 2B). The BRCA2 mutant OCIP232 and OCIPA1 models also showed prolonged growth inhibition and survival in response to either treatment ($P<0.0001$). Neither treatment led to significant changes in body weight (Supplementary Figure 2).

BRCA1/2 mutation result in the accumulation of DNA damage. Tumours treated with a single dose of cisplatin or vehicle were stained for γH2AX and CC3 to examine DNA damage and apoptosis in response to treatment. Tumours were excised 24 h after treatment. The BRCA mutant xenografts (OCIP28, OCIP217, OCIP232 and OCIPA1) showed a significant accumulation of DNA damage ($P<0.0001$) as measured by γH2AX staining that was not observed in the control xenografts (OCIP19, OCIP23 and OCIP167; Figure 3A). Even though the BRCA2 mutant xenograft models showed a significant accumulation of DNA damage, no significant increase in CC3 staining was observed ($P=0.57$; Figure 3B). This was also observed in OCIP217. Alternatively, the control tumours (OCIP19, OCIP23 and OCIP167) displayed a mild but nonsignificant increase in CC3 staining ($P=0.58$) in response to treatment with cisplatin.

BRCA1 and BRCA2 patients. All the four BRCA mutant xenografts were derived from patients whose mutation was known before implantation, and their clinical features, response to treatment and survival are summarised in Table 1 and Table 2. All of these patients responded to platinum-based chemotherapy. The donor of OCIP28 presented with an unresectable tumour owing to involvement of the superior mesenteric artery. There was a major response to cisplatin combined with gemcitabine, and the patient was then able to undergo curative-intent surgery, (followed by postoperative treatment with radiotherapy and capecitabine and remains alive more than 5 years after surgery. The patient from which OCIP217 was derived showed a complete and sustained response of a solitary liver metastasis following cisplatin plus gemcitabine treatment (no regrowth once chemotherapy was discontinued for 6 months) and a major response of the primary tumour (Figure 2C). She subsequently underwent distal pancreatectomy and wedge resection at the site of the liver lesion (no viable

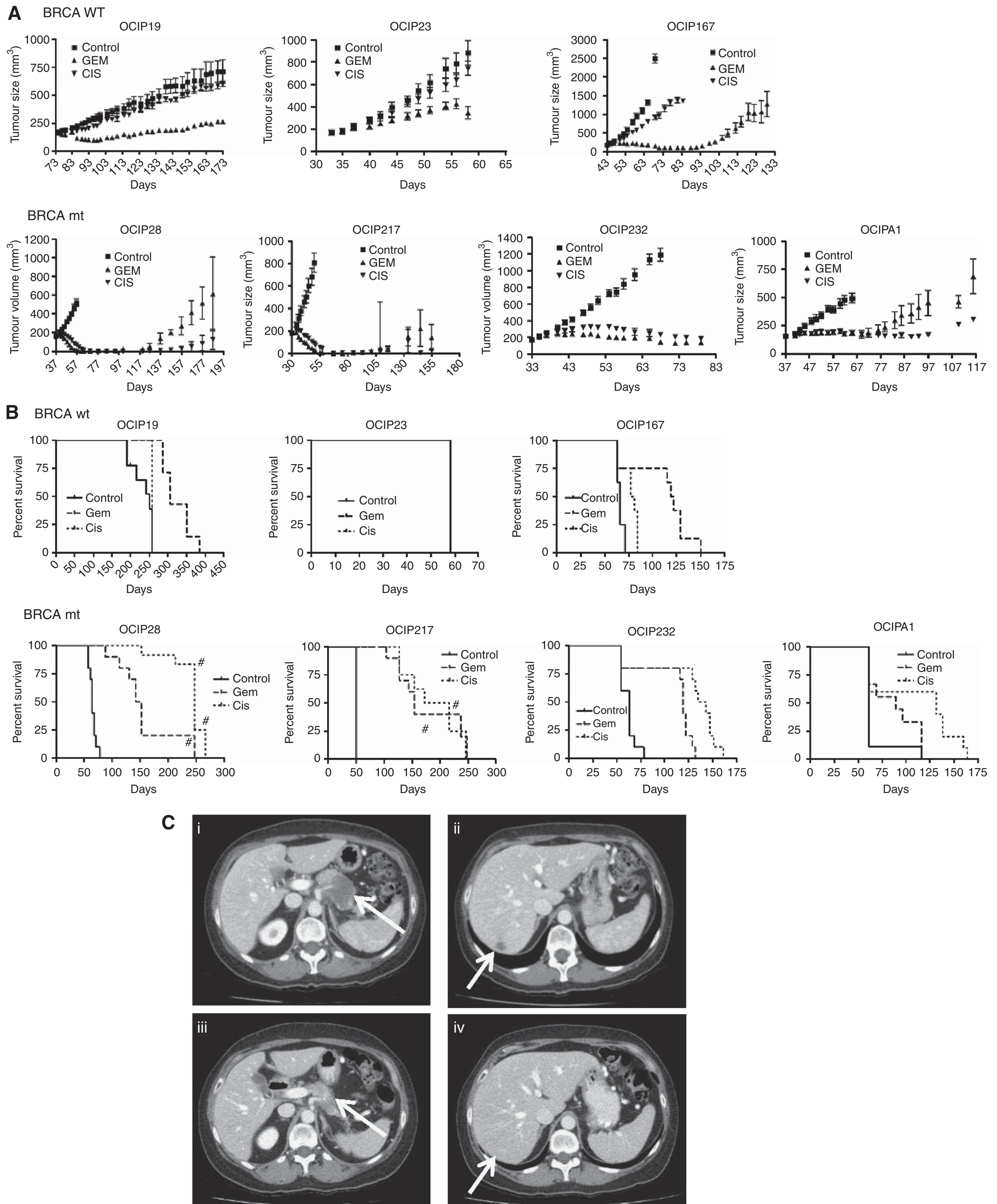

Figure 2. Mutations in BRCA1 and BRCA2 increase treatment sensitivity and survival. (**A**) Mice were treated for 4 weeks with cisplatin, gemcitabine or vehicle, and tumour volume was evaluated three times a week; mt, mutant. (**B**) Survival in response to treatment was measured until mice either reached the humane tumour end point or their natural life span in the case of no recurrence of the tumour after treatment. Error bars represent s.d. # indicates no tumours detected. Mice were killed due to old age. (**C**) CT scans for patient from which OCIP217 was derived. Primary tumour in the body of pancreas with involvement of coeliac axis and splenic artery (i, arrow) and solitary liver metastasis (ii, arrow). Major response to gemcitabine and cisplatin with marked shrinkage of primary tumour (iii, arrow), and radiologic complete response of liver metastasis (iv, arrow).

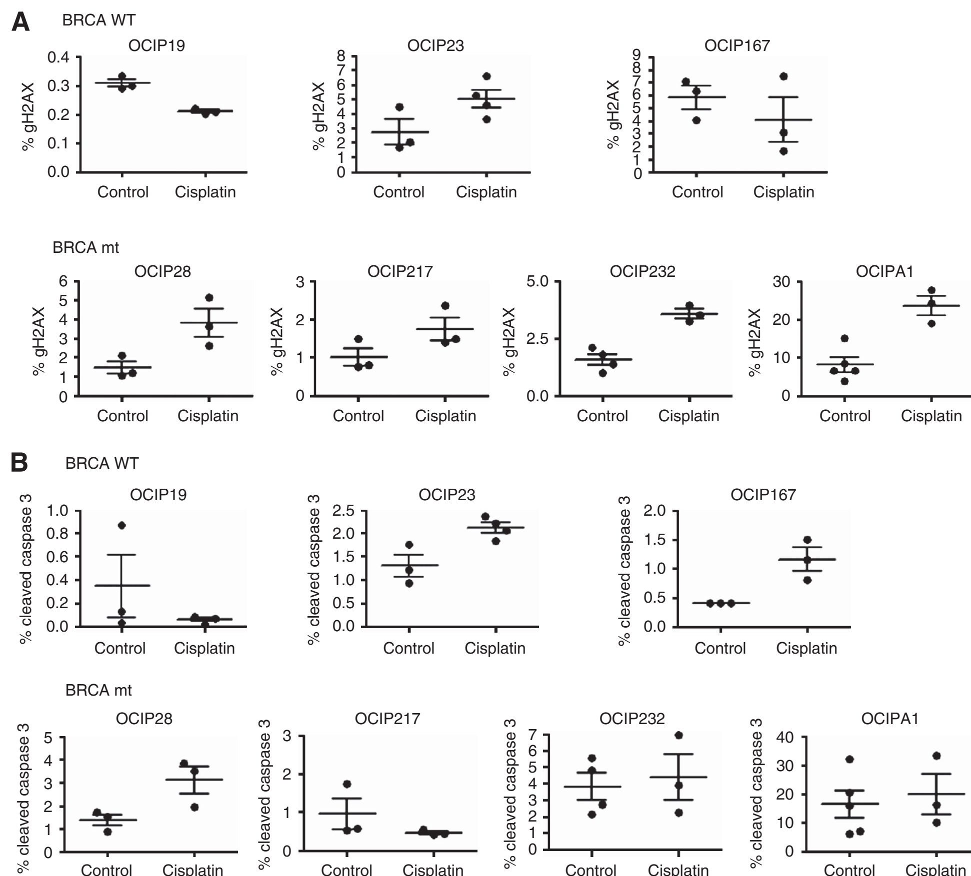

Figure 3. **BRCA1 and BRCA2 mutations result in γH2AX foci accumulation in response to cisplatin treatment.** Section of mice treated with a single dose of cisplatin or vehicle were stained for (**A**) γH2AX and (**B**) CC3. BRCA mutant (mt) models display a significant increase in γH2AX foci that was not observed in the WT models 24 h after treatment. Cleaved caspase-3 staining, however, was not increased in the BRCA mt models. Error bars represent s.d.

Table 2. Treatment response

		Treatment	
Model	RT	Chemo	Response
OCIP19	Y	Gem[a]	No evidence of disease
		Gem–RT[a]	No evidence of disease
OCIP23	N	Gem[a]	Disease progression
OCIP28	Y	Gem–Cis[b]	Partial response
		Capecitabine[a]	No evidence of disease
OCIP167	N	Gem[a]	No evidence of disease
		5FU–irinotecan–oxaliplatin[c]	Stable disease
OCIP217	Y	Gem–Cis[b]	Partial response
		Gem–Cis[c]	Stable disease
		Carboplatin–paclitaxel	Disease progression
OCIP232	N	Gem[a]	No evidence of disease
		5FU–irinotecan–oxaliplatin–leucovorin[c]	Initial response, progressed after 6 months
		Veliparib[c]	Disease progression
OCIPA1	Y	Gem[b]	Disease progression
		Gem–Cis[c]	Mixed response (primary: response; liver met: progression)

Abbreviations: Cis = cistplatin; Gem = gemcitabine; N = no; RT = radiotherapy; Y = yes.
[a]Adjuvant.
[b]Neoadjuvant.
[c]Palliative.

tumour was found in the liver wedge), but developed peritoneal metastases 3 months later. These were initially controlled using cisplatin plus gemcitabine, but the disease then became platinum refractory and the patient died. The donor of OCIP232 underwent Whipple resection followed by adjuvant gemcitabine, but developed liver metastases less than 1 year post surgery. He was then treated using the FOLFIRINOX protocol (5-fluorouracil, irinotecan and oxaliplatin), showed an initial response, but the disease became refractory after 8 months. The patient was briefly enrolled in a clinical trial of the PARP inhibitor velaparib, but came off study after 2 weeks because of declining performance and died. The donor of OCIPA1 presented with locally advanced disease, showed an initial response to gemcitabine chemoradiation, but then developed malignant ascites (from which the xenograft was established) and osteoblastic metastases. Treatment was changed to cisplatin plus gemcitabine upon identification of a germline BRCA2 mutation. The patient experienced a dramatic but short-lived clinical response, with resolution of ascites and pain, but then developed leptomeningeal metastases and died 4 months later. Because of the difference in grade, clinical stage and treatments given in this small patient series, we did not attempt a statistical analysis for the correlation of xenograft and patient data or treatment efficacy.

DISCUSSION

Germline mutations in BRCA1 and BRCA2 result in defects in BRCA-mediated HR, which is important for the maintenance of

genome stability (Venkitaraman, 2001; Gudmundsdottir and Ashworth, 2006). These mutations predispose to breast and ovarian cancers (Foulkes and Shuen, 2013; Kobayashi *et al*, 2013), and are associated with increased risk of pancreatic cancer (Fernandes *et al*, 1994; Klein *et al*, 2001; Bartsch *et al*, 2004; Grant *et al*, 2014). In addition to a role in tumour development, BRCA1 and BRCA2 mutations are associated with sensitivity to platinum drugs and other DNA-damaging agents in ovarian cancers (Dann *et al*, 2012; Muggia and Safra, 2014), and there are anecdotal reports that this is also the case in pancreatic cancers (Lowery *et al*, 2011; Sonnenblick *et al*, 2011). Additional support for this comes from a recent retrospective study of 71 pancreatic cancer patients with germline BRCA1 and BRCA2 mutations that reported superior overall survival for the patients who were treated with platinum, compared with those who were not treated with platinum (Golan *et al*, 2014). However, the clinical development of treatment protocols for these patients is hampered by their relative rarity. To address this, we studied cisplatin activity in a series of primary xenografts obtained from platinum-naive pancreatic cancer patients, compared with a matched group of BRCA WT control tumours, and we made a number of novel observations.

With the exception of the BRCA2 mutant xenograft OCIP28, in which there was loss of heterozygosity, all the four xenografts derived from patients with germline BRCA mutations maintained the genetic features seen in the donor. They displayed a range of phenotypic features that have been linked to aggressive growth, including doubling time, stroma density and hypoxia that were similar to the control group of primary xenografts that lacked BRCA mutations. Importantly, RAD51 repair foci were observed in the control tumours 24 h after cisplatin treatment, but not in the four BRCA mutant xenografts, confirming their functional deficiency in DSB repair.

As expected, all the four BRCA mutant models were sensitive to cisplatin, which produced long-term control of established OCIP28 and OCIP217 tumours. Interestingly, these tumours also showed comparable sensitivity to gemcitabine. In contrast, cisplatin was essentially inactive against the BRCA WT xenografts, although gemcitabine produced some degree of growth inhibition in all the three models, with pronounced tumour shrinkage and prolonged survival in OCIP167.

This is consistent with observations recently published by Waddell *et al* (Waddell *et al*, 2015) who analysed whole genomes of 100 pancreatic ductal adenocarcinomas. Similar to the results presented in this study, PDX models carrying a germline BRCA2 mutation showed high sensitivity to treatment with both cisplatin and gemcitabine. Although Waddell *et al* used both agents at their maximum-tolerated dose, we used significantly lower doses in our treatments (140 mg kg^{-1} *vs* 100 mg kg^{-1} gemcitabine, 6 mg kg^{-1} *vs* 4 mg kg^{-1} cisplatin). Similar growth delay in response to treatment was observed in both studies despite the difference in dose, suggesting that the dose can be adapted to reduce side effects without loss of treatment efficacy.

It has been proposed that the typically dense stroma of pancreatic cancer affords a barrier to drug penetration (Olive *et al*, 2009), although this was not supported by recent studies (Rhim *et al*, 2014; Sherman *et al*, 2014). There was no obvious correlation between stroma density and treatment sensitivity in these models.

The differential sensitivity of the BRCA WT xenografts to gemcitabine and cisplatin is consistent with gemcitabine as the more clinically active drug. In contrast, all the four BRCA mutant xenografts were highly sensitive to both drugs, with long-term complete regression of established tumours seen in some cases following treatment with either agent. This finding confirms the clinical impression that pancreatic cancers in patients with germline BRCA mutations may be unusually sensitive to platinum-containing compounds. Less attention has been paid to their sensitivity to gemcitabine, which also causes DSBs (Plunkett *et al*, 1995; Jones *et al*, 2014). Within the constraints of the small sample size, there was no correlation between tumour growth rate, stroma density and the level of hypoxia and response to either drug.

All the four patients with germline BRCA mutations had the mutation identified before the establishment of the xenografts and treatment with platinum. The response of the xenografts roughly tracks that seen in the corresponding patient, with the donors of the two most-responsive xenografts, OCIP28 and 217, both achieving downstaging with gemcitabine plus cisplatin chemotherapy to undergo curative-intent surgery. It is noteworthy that the donor of OCIP28 remains disease-free after more than 5 years, whereas that of OCIP217 showed complete response of a solitary liver metastasis and over 50% shrinkage of a 6-cm tumour in the body of the pancreas. In contrast, the donor of the least-responsive xenograft, OCIPA1, rapidly developed cisplatin resistance and survived for only 4 months. With the exception of the OCIP28 donor, resistance to platinum-containing treatment developed in all the patients. It can be speculated that this occurred, in part, due to the emergence of a clone of HR-proficient cancer cells, or to unrelated mechanisms of acquired drug resistance involving, for example, drug accumulation or detoxification pathways.

In summary, we have characterised a panel of primary xenografts derived from pancreatic cancer patients carrying germline BRCA mutations, and have shown that their genetic features and platinum sensitivity closely recapitulate those seen in the patient donor. These models also show greater sensitivity to gemcitabine compared with a matched series of BRCA WT tumours, which we believe is important and under-appreciated when considering future trials of drug combinations. These findings support ongoing preclinical research using these models to identify mechanisms for the emergence of cisplatin resistance in BRCA mutant pancreatic cancers, as well as the evaluation of treatment protocols that incorporate additional agents such as PARP inhibitors.

ACKNOWLEDGEMENTS

This work was supported by the Farb Family Fund, Princess Margaret Cancer Foundation, the Ontario Ministry of Health and Long-Term Care (OMHLTC) and grants from the Ontario Institute for Cancer Research and the Canadian Cancer Society Research Institute (CCSRI). The views expressed do not necessarily reflect those of the OMHLTC.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)