BRCA1/2 MUTATION ANALYSIS IN 41 OVARIAN CELL LINES REVEALS ONLY ONE FUNCTIONALLY DELETERIOUS BRCA1 MUTATION

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Running Title – BRCA1/2 mutations in ovarian cancer cell lines.

Abstract

Mutations in BRCA1/2 increase the risk of developing breast and ovarian cancer. Germline BRCA1/2 mutations occur in 8.6-13.7% of unselected epithelial ovarian cancers, somatic mutations are also frequent. BRCA1/2 mutated or dysfunctional cells may be sensitive to PARP inhibition by synthetic lethality. The aim of this study is to comprehensively characterise the BRCA1/2 status of a large panel of ovarian cancer cell lines available to the research community to assist in biomarker studies of novel drugs and in particular of PARP inhibitors.

The BRCA1/2 genes were sequenced in 41 ovarian cell lines, mRNA expression of BRCA1/2 and gene methylation status of BRCA1 was also examined. The cytotoxicity of PARP inhibitors olaparib and veliparib was examined in 20 cell lines.

The cell line SNU-251 has a deleterious BRCA1 mutation at 5564G>A, and is the only deleterious BRCA1/2 mutant in the panel. Two cell lines (UPN-251 and PEO1) had deleterious mutations as well as additional reversion mutations that restored the protein functionality. Heterozygous mutations in BRCA1/2 were relatively common, found in 14.6% of cell lines. BRCA1 was methylated in two cell lines (OVCAR8, A1847) and there was a corresponding decrease in gene expression. The BRCA1 methylated cell lines were more sensitive to PARP inhibition than wild-type cells. The SNU-251 deleterious mutant was more sensitive to PARP inhibition, but only in a long-term exposure to

correct for its slow growth rate. Cell lines derived from metastatic disease are significantly more resistant to veliparib (2.0 fold p = 0.03) compared to those derived from primary tumours. Resistance to olaparib and veliparib was correlated Pearsons-R 0.5393, p = 0.0311.

The incidence of BRCA1/2 deleterious mutations 1/41 cell lines derived from 33 different patients (3.0%) is much lower than the population incidence. The reversion mutations and high frequency of heterozygous mutations suggest that there is a selective pressure against BRCA1/2 in cell culture similar to the selective pressure seen in the clinic after treatment with chemotherapy. PARP inhibitors may be useful in patients with BRCA1 deleterious mutations or gene methylation.

Key words – BRCA1/2, ovarian, mutation, methylation, parp inhibitor, olaparib, veliparib

Introduction

The BRCA1 and BRCA2 proteins play a critical role in DNA damage repair and mutations in these genes increase the risk of developing breast and ovarian cancer. BRCA1/2 germline mutations have been shown occur in 8.6-13.7% of unselected epithelial ovarian cancer patients (Pal et al. 2005; Risch et al. 2001; Rubin et al. 1998). The rates of BRCA1 or combined BRCA1/2 mutation are much higher in ovarian cancer than in unselected invasive breast cancer 1.42-1.96% (2000; Newman et al. 1998; Papelard et al. 2000).

Synthetic lethality is the phenomenon whereby cell death results from the loss of function of two different gene products, when loss of either gene product in isolation does not. This concept has led to the idea that a new class of agents, PARP inhibitors, could be effective at treating BRCA1/2 dysfunctional cancers. PARP-1 is thought to be a regulator of base excision repair (BER) (Helleday 2011). One model suggests that PARP-1 inhibition traps one of the single strand break intermediates in the BER process and prevents the final ligation step (Helleday 2011). PARP also plays a role at stalled replication forks and is often hyper-activated in cells defective in homologous recombination repair such as BRCA1/2 mutant or defective cells (Helleday 2011). Inhibition of PARP in cells with dysfunctional homologous recombination repair therefore results in the inhibition of two major DNA repair pathways. Studies have demonstrated this synthetic lethality by inhibiting PARP in cells deficient in BRCA1/2 (Bryant et al. 2005; Farmer et al. 2005). The results of proof of concept clinical trials of

the PARP inhibitor olaparib in breast and ovarian cancer patients with germline BRCA1/2 mutations have also been encouraging (Audeh et al. 2010; Tutt et al. 2010).

Recently it has been shown that somatic BRCA1/2 mutations are also frequent in ovarian cancer. BRCA1/2 mutations were detected in 18.3% of tumour samples (n=235). Of the 42 patients found to have a BRCA1/2 mutation in their tumor, germline DNA was available from 28. Eleven of these patients (39%) had somatic BRCA1/2 mutations not present in the germline (Hennessy et al. 2010). These somatic BRCA1/2 mutations may expand the cohort of ovarian cancer patients which may benefit from treatment with PARP inhibitors. PARP inhibitors may also be useful in sporadic ovarian cancer patients which have other non-mutation defects in the BRCA1/2 pathways in their tumours, such as loss of gene or protein expression which can occur in up to 90% of patients (Russell et al. 2000).

To understand the role of BRCA1/2 mutations in ovarian cancer and study potential therapeutic strategies including the use of PARP inhibitors, suitable cell lines are needed. A previous study has characterised BRCA1/2 status in a large panel of breast cancer cell lines (Elstrodt et al. 2006). Our study characterises the mutation, expression and methylation status of BRCA1/2 in 41 ovarian cancer cell lines. The responsiveness to PARP inhibitors olaparib and veliparib was examined in a subset of 20 cell lines.

Results

BRCA1 Mutation Screening

The clinical characteristics of the ovarian tumors from which the cell lines were established are presented in Table 1. Sequencing of BRCA1 revealed 18 different alterations in the gene sequence among 41 human ovarian cell lines (Table 2). The majority of alterations are nonpathogenic polymorphisms as described by the Breast Cancer Information Core (BIC) mutation database (http://research.nhgri.nih.gov/bic/). Three novel mutations in BRCA1 were identified in this study 1199del29, 2921 G>A, and 2925 G>A (Table 3). These variants have not been previously identified and are not present in the BIC or Myriad Genetics databases. 1199del29 is deleterious as it results in a frameshift and a premature stop codon; 2132 G>A is a polymorphism and 2136 G>A is a variant of unknown significance.

Deleterious BRCA1 mutations were identified in 4 ovarian cell lines IGROV-1, UPN-251, HI0180 and SNU-251 (Tables 2 and 3). The deletion of an adenine at position 2080 in the IGROV-1 cell line, leads to a premature stop of protein translation at amino acid 700. However, IGROV-1 is heterozygous not homozygous for the 2080delA BRCA1 mutation (Figure 1A) as previously reported (Foray et al. 1999). The germline genotype of BRCA1 in the patient who IGROV-1 was derived from is unknown, so we cannot determine if this mutation is germline or somatic (Benard et al. 1985; Fajac et al. 1996). The immortalised ovarian epithelial cell line HI0180 (Sood et al. 2002) has a suspected

deleterious mutation at IVS21+1G>C, the first base after exon 21. However, this mutation is also heterozygous.

The 1199del29 mutation in BRCA1 is defined as deleterious as it results in a frameshift causing a premature stop codon. UPN-251 has the 1199del29 mutation however there is an additional mutation (1246delA), which restores the reading frame of the protein (Figure 1C). Therefore, despite having two mutations that would independently be deleterious, the UPN-251 cell line likely has a functional BRCA1 due to the combination of the two mutations. We believe this is an example of a deleterious mutation, followed by a reversion mutation.

SNU-251 has a homozygous deleterious mutation at 5564G>A which converts a tryptophan to a stop codon truncating the protein in exon 23. SNU-251 was developed from malignant ascites fluid from a Korean patient with endometrioid ovarian carcinoma (Yuan et al. 1997). The germline BRCA1 status of this patient is unknown.

BRCA2 Mutation Screening

Sequencing of BRCA2 revealed 33 different alterations in the gene sequence among 43 human ovarian cell lines (Tables 3 and 4). Similar to BRCA1 the majority of alterations are nonpathogenic polymorphisms. Five novel mutations in BRCA2 were identified in this study (Table 3). 3624A>G, 4542C>T, 4680C>T are polymorphisms. 5192A>T and 5193C>T are reversion mutations correcting a deleterious mutation, explained below.

Deleterious BRCA2 mutations were identified in 5 ovarian cell lines IOSE80, OC316, OVCA432, OVCA433 and PEO1 (Tables 3 and 4). The deletion of an adenine at position 5579 in the OC316 cell line, leads to a premature stop of protein translation at amino acid 1790. However, OC316 is heterozygous, not homozygous for the 5579delA BRCA2 mutation. The germline genotype of BRCA2 in the patient who OC316 was derived from is unknown (Alama et al. 1996). The deleterious mutations in IOSE80, OVCA432, and OVCA433 are also all heterozygous.

The PEO1 cell line has a BRCA2 homozygous mutation 5193C>G, which would normally result in a stop codon at amino acid 1655. However, the PEO1 cell line has another mutation 5192A>T which prevents the stop codon. We believe that this is an example of a reversion mutation. The cell lines PEO4 and PEO6 were established from the ascites fluid of the same patient as PEO1 after further treatment with chemotherapy (Langdon et al. 1988). The PEO4 and PEO6 cells have a different mutation at 5193, it is now a thymine and the 5192A>T mutation seen in PEO1 is gone. This appears to be a different reversion mutation at the same site.

LOH, methylation and QPCR

Loss of heterozygosity (LOH) was common at both the BRCA1 and BRCA2 locus (Table 3). Out of the 34 cell lines examined 15 had a LOH at BRCA1 and 10 had a LOH at BRCA2 (44% and 29% respectively). Of the 6 cell lines with heterozygous deleterious mutations, 5 were examined for LOH. All of these cell lines (IGROV-1, IOSE80, OC316, OVCA432, and OVCA433) did not show LOH at the respective BRCA1/2 locus.

Methylation was examined on the BRCA1 gene in all 41 cell lines and was found in 2 cell lines A1847 and OVCAR8 (Table 3). The methylated cell lines had a corresponding decrease in gene expression of BRCA1 (Figure 2). The deleterious mutated cell line SNU-251 had similar BRCA1 gene expression to the wild-type cell lines. The premature stop codon results from a single base change and this does not have an impact on the length of the mRNA produced.

Cytotoxicity and growth assays

A panel of 20 cell lines was chosen to examine the relationship between BRCA1 mutation, methylation and resistance to PARP inhibition. The following cell lines were examined, deleterious mutation (SNU-251), methylated (OVCAR8 and A1847) and 17 wild-type cell lines. The IC50s of olaparib in the cell line panel is shown in Figure 3A. The methylated cell lines OVCAR8 and A1847 were more sensitive than the wild-type cells on average to olaparib supporting the synthetic lethality theory between BRCA1 dysfunction and PARP inhibition (Figure 3B). Unexpectedly, SNU-251, the mutated cell line, was the second most resistant to olaparib. The SNU-251 cells were the slowest growing cell line in the panel tested with a doubling time of 3.32 ± 0.85 days (Figure 3C). In the 7-day cytotoxicity assay the SNU-251 cells only divide twice, the other cell lines divide 3-4 times. The methylated cell lines are on average slower growing than wild-type cells but this difference was not significant (Figure 3D). To compensate for the slower growth rate the SNU-251 cells were examined with a 2 week cytotoxicity assay. This longer exposure time gives an equivalent number of cell doublings to the rest of the cell

panel in a 1 week assay. SNU-251 with a 2 week exposure is relatively sensitive to olaparib (Figure 3A & 3B).

The results for veliparib were similar (Figure 3E); however a higher dose of veliparib was needed than olaparib to achieve an IC50 across the cell line panel. The IC50s of olaparib and veliparib are also correlated in the panel, Pearsons-R 0.5393, p = 0.0311. The methylated cell lines OVCAR8 and A1847 were also on average more sensitive to veliparib than wild-type cells (Figure 3F). SNU-251 was also relatively resistant to veliparib in a 1 week cytotoxicity assay and more sensitive in a 2 week assay. Two cell lines were relatively sensitive to both olaparib and veliparib (A2780 and ES-2), and OVCAR420 was highly resistant to both agents. However, the next most resistant cell lines were different for olaparib (OVCAR432, HEY, IGROV-1) compared to veliparib (SKOV3, UPN-251).

T-tests were used to analyse if the clinical characteristics of the patients the cell lines were derived from were related to the response of the cell lines to parp inhibitors (Table 5). Cell lines derived from primary tumors cells were significantly more sensitive to veliparib (p = 0.03) than cells from metastases, including A2780 and ES-2 cells. The response to olaparib was not significant as the cell line IGROV-1 responded differently to A2780 and ES-2 and was more resistant to Olaparib. Cell lines which were derived from patients after receiving chemotherapy tended to be more resistant to veliparib than cells derived after chemotherapy, 1.83-fold p = 0.08. All other factors were non-significant in this relatively small sample set.

Discussion

When BRCA1/2 mutations are detected in cell lines they represent either a germline mutation or a somatic mutation in the tumour, or a combination of both. Currently in clinical practice BRCA1/2 mutations are screened for in the germline with a blood test. This test is more economical, does not require tumor tissue and addresses the issue of familial risk of breast and ovarian cancer. However, with the advent of PARP inhibitors as a potential targeted therapy for BRCA1/2 mutated cancer additional tumour testing for somatic mutations may become a new standard as a significant number of somatic mutations are present in ovarian cancers (Hennessy et al. 2010).

The results of this study show that deleterious BRCA1/2 mutations are rare in ovarian cancer cell lines, only 1/41 cell lines studied (2.4%). The clinical characteristics of the patients the ovarian cell lines were derived from is presented in Table 1. Five cell lines in the panel represent later stages of cancer progression in patients cell lines were already derived from (HOC-7, PEA2, PEO4, PEO6, PEO14). Three cell lines are immortalised ovarian epithelial cells, HIO180, IOSE29 and IOSE80. Therefore, our panel of 41 cell lines represents 33 different ovarian cancer patients and the BRCA1/2 deleterious mutation rate per patient the cell lines were derived from is 1/33 (3.0%).

The histology of the cell line panel (Table 1) is reflective of clinical ovarian cancer; only one cell line is of non-epithelial origin, PA-1 which was derived from a germ-cell tumour (Hills et al. 1989). Therefore 32/33 patients that the cell lines were derived from had

epithelial ovarian cancer (97.0%), which is similar to the reported clinical rates of epithelial ovarian cancer (~90.0%) (Colombo et al. 2009). Out of the patients where the subtype of epithelial ovarian cancer is known (20), serous dominates over other epithelial subtypes in the cell line panel 14/20 (70.0%), slightly lower than what is seen in the clinic (~80-85%) (Colombo et al. 2010). This discrepancy is likely due to many cell lines being classified as adenocarcinoma, rather than into epithelial subgroups. Interestingly, although the majority of BRCA1 mutations occur in serous ovarian cancer (Berchuck et al. 1998; Lakhani et al. 2004) the SNU-251 BRCA1 mutant is derived from an endometrial ovarian tumour (Yuan et al. 1997). The PEO1, PEO4 and PEO6 cell lines which have reversions of a BRCA2 mutation are of also of serous origin from the same patient (Langdon et al. 1988)

Deleterious BRCA1 mutations have also been shown to be rare in breast cancer cell lines, 3/41 (7.3%) (Elstrodt et al. 2006). However, due to the higher reported rates of deleterious BRCA1/2 mutations in unselected invasive ovarian versus unselected invasive breast cancer patients (11.7-15.3% versus 1.71%) (Pal et al. 2005; Papelard et al. 2000; Risch et al. 2001; Rubin et al. 1998), one would expect to see a higher number of cell lines with mutations in ovarian cancer when comparing unselected cell-line panels of similar size. This is in contrast to what we have observed; this discrepancy may be in part due to random sampling, the cell models we have studied may have been derived more often from patients without BRCA1/2 mutations; or there may be factors selecting against these mutations during adaption to cell culture.

Unusually, heterozygous mutations in BRCA1/2 were seen in 4 ovarian cancer cell lines studied (IGROV-1, OC316, OVCAR432 and OVCAR433), representing a frequency of 4/33 (12.12%). Heterozygous mutations in BRCA1/2 were also seen in two of the immortalised ovarian cell lines IOSE80 and HI0180. LOH was studied in all of the heterozygous mutated cancer cell lines and no LOH were observed (Table 3). One explanation for why heterozygous BRCA1/2 mutations may be observed in a cancer cell line is the cancer patient the cells were derived from was a BRCA1/2 mutation carrier but a second mutation in BRCA1/2 was not part of the carcinogenesis of their tumour. Breast cancers in patients who are heterozygous for BRCA1/2 are rarely ER+. However, there is a subset of patients that are both (Foulkes et al. 2004; Tung et al. 2010b), these patients may be ER+ because their cancer occurred due to hormonal carcinogenesis rather than loss of the second BRCA1/2 allele. One study examined 77 breast cancer patients women with BRCA1 germline mutations, 12 tumours remained heterozygous (15.5%), and 8 of these were ER+ (Tung et al. 2010a). It appears that heterozygous BRCA1/2 mutations may be more common in breast cancer where the lifetime risk of sporadic disease is higher than ovarian cancer, 1 in 8 vs. 1 in 72 (National Cancer Institute. 2010). In contrast to our observations with cell lines, of the BRCA1/2 mutant ovarian tumours identified to date by Myriad Genetics, only ~1/100 is heterozygous (Timms 2011).

When BRCA1/2 mutated cells are treated with chemotherapeutics, one mechanism of resistance that can occur is a reversion of the deleterious mutation. This has been shown to occur in cancer cell lines (Sakai et al. 2008) as well as in cancer patients after treatment with platinum-based chemotherapy (Swisher et al. 2008). This demonstrates

that when the selective pressure of drug treatment is applied to BRCA1/2 mutated cells a common occurrence is a reversion of the mutation. The development of cell lines from tumour tissue is a process that involves multiple levels of selective pressure. Primary culture protocols involve physical disruption of the tissue, enzymatic digestion and selection of attached colonies in tissue culture. The more robust the cell, the more likely it is to survive the process. Cells without the full complement of DNA repair pathways, such as BRCA1/2 mutants may be at a disadvantage.

Loss of the second BRCA1/2 allele has also been shown to be heterogeneous within a tumour from BRCA1/2 mutation carriers (Martins et al. 2012). Mutations in BRCA1, PTEN and p53 were analysed at the single cell level in 55 BRCA1-associated breast tumours. Based on the frequency of each mutation loss of PTEN was the most common 'first' carcinogenic event associated with a basal-like subtype rather than BRCA1 (Martins et al. 2012). When a cell line is isolated from a heterogeneous tumour from a BRCA1/2 carrier cells with PTEN and p53 mutations could have been more robust in culture and BRCA1/2 mutated cells did not survive in the established cell line.

The heterozygous BRCA2 mutated cell line OC316 was derived from the malignant ascites of a cancer patient who had already received multiple rounds of chemotherapy (Alama et al. 1996) (Table 1). The metastasis and treatment with chemotherapy may have provided enough selective pressure for BRCA2 heterozygous cells to dominate the population. IGROV-1 was derived from an untreated primary tumour (Benard et al. 1985) and its heterozygosity may be explained by an alternate path of tumorigenesis. IGROV-1

is PTEN null (Cruet-Hennequart et al. 2003) but p53 wild-type (Casalini et al. 2001). It is possible that the primary tumour IGROV-1 was derived from was a mixture of PTEN and BRCA1 mutant cells and the PTEN mutation survived the selective pressure of cell culture.

The reversion mutations in UPN-251, PEO1, PEO4 and PEO6 are also evidence of a selective pressure against BRCA1/2 mutations. In the PEO4 and PEO6 cells, the reversions occurred after the patient received further chemotherapy (Langdon et al. 1988). In the PEO1 cells, we see an additional variation compared to that reported by Sakai *et al* (Sakai et al. 2009), 5192>T which has been previously reported by Stronach et al and has been seen in other unselected stocks of PEO1 (Stronach et al. 2011). Our PEO1 cells were of relatively high passage number (p119) at the time of analysis for BRCA1/2 mutation. Stronach *et al* hypothesise that 5192>T exists as a sub-dominant population within the original PEO1 line given that the same mutation has been reported to emerge independently either following selection with cisplatin (Sakai et al. 2009) or spontaneously following long term cell culture. It has also been shown that PEO4 and PEO6 are genetically diverse from PEO1, consistent with the selection of a pre-existing resistant subclone rather than the transformation of PEO1 into PEO4/6 (Cooke et al. 2010). In the UPN-251 cells, we don't know if these reversions occurred *in vivo* or *in vitro*. However, the UPN-251 cells were derived from a patient who had failed frontline platinum/taxol as well as salvage taxol, which may have provided the selective pressure for the reversion (Hamilton 2012).

The scientists who established SNU-251 commented on the difficulty developing cell lines from ovarian cancer patients, their success rate was only 17% (Yuan et al. 1997). Another BRCA1-mutated ovarian cell line (UWB1.289) was developed from a patient with a particularly aggressive cancer (DelloRusso et al. 2007). The authors of this study commented on the difficulty of establishing BRCA1 mutated cell lines in cell culture and speculate that the aggressiveness of this patient's tumour may have facilitated the propagation of this particular deleterious mutated cell line *in vitro* (DelloRusso et al. 2007).

An unselected breast cancer cell-line panel was skewed towards the triple-negative subgroup, 21/48 cell lines studied (43.75%) (Kao et al. 2009), higher than the incidence of triple negative breast cancer (TNBC) in unselected invasive breast cancer (12.4%) (Bauer et al. 2007). One would expect that BRCA1 mutations would be enriched in the panel as BRCA1 mutated tumours are common in TNBC (20.93%) (Merkel et al. 1989). However, BRCA1 mutations were relatively uncommon 2/14 (14.2%) of the TNBC cell lines examined (Kao et al. 2009). This suggests that the clinical aggressiveness of TNBC may make development of cell lines easier in the laboratory, but that the presence of BRCA1/2 mutations hinders the development of cell lines, resulting in a bias in the available cell lines.

The SNU-251 BRCA1 deleterious mutated cell line was relatively resistant to PARP inhibition in a 1 week cytotoxicity assay (Figure 3A,E). This is the opposite of what was predicted by the synthetic lethality theory between PARP inhibition and BRCA1/2 dysfunction. The SNU-251 cell line is likely to have many changes in its DNA repair

mechanisms in addition to the BRCA1 mutation and more detailed study is needed to understand any DNA repair defects present. The deleterious mutation is also located near the end of the gene sequence and may have little impact on protein function. However, SNU-251 has a very slow growth rate compared to the other cell lines in the panel (Figure 3C). One of the causes of toxicity from PARP inhibitors is the generation of DSBs at collapsed replication forks (Rouleau et al. 2010). It is possible that the slow growth rate masks the true sensitivity of the cell line as the cells do not go through as many cell divisions as their wild-type counterparts. When SNU-251 was examined with a 2-week cytotoxicity assay their sensitivity to both olaparib and veliparib increases and they are more sensitive than wild-type cells.

The rate of BRCA1 methylation in the cell line panel 2/33 different patients (6.0%) is similar to the observed rate clinically in ovarian tumours 12/98 (12.2%) (Baldwin et al. 2000). BRCA1 methylation status has been shown to be stable in recurrent ovarian cancers (Baldwin et al. 2000). The BRCA1-methylated cell lines OVCAR8 and A1847 were more sensitive to PARP-inhibition than wild-type cell lines (Figure 3B, F). These cells also had a lower expression of BRCA1 mRNA (Figure 2). This data supports the synthetic lethality theory between PARP inhibition and BRCA1 dysfunction. This observation needs to be expanded upon but examining BRCA1 methylation may be relevant to predicting sensitivity to PARP inhibitors.

Two BRCA1/2 wild-type unmethylated cell lines were relatively sensitive to both olaparib and veliparib (A2780 and ES-2). This demonstrates that parp inhibitors may be

useful for the treatment of a broader spectrum of patients with ovarian cancer. Cells lines derived from metastases were significantly more resistant to veliparib than cells derived from primary tumours (Table 5). This is consistent with the concept that pathways that govern invasion and metastasis overlap with drug resistance (Liang et al. 2002). It is interesting that this observation is only the case for veliparib and not olaparib, suggesting a divergent mechanism of resistance between the two parp inhibitors. Some of the most resistant cell lines were different for olaparib (OVCAR432, HEY, IGROV-1) compared to veliparib (SKOV3, UPN-251). One possible explanation for this difference in resistance mechanisms may be due to the expression of ABC transporters. There has been some evidence for olaparib being a P-glycoprotein substrate in an animal model (Rottenberg et al. 2008) and for veliparib not being a P-glycoprotein substrate in a transfected cell line (Li et al. 2011).

Some studies have suggested that BRCA1/2 heterozygosity has a distinct phenotype and is associated with increased sensitivity to DNA damaging agents (Warren et al. 2003). Other studies have shown no difference in DNA repair capacity in response to radiation in BRCA1/2 heterozygotes (Nieuwenhuis et al. 2002). In this study we found no difference in the response of the BRCA1/2 +/- cell lines to the parp inhibitors olaparib and veliparib compared to wild-type cells (Table 5).

Conclusions

We provide herein detailed BRCA1/2 status in a large panel of ovarian cancer cell lines to facilitate in vitro studies of parp inhibitors and other DNA damaging agents. The incidence of BRCA1/2 deleterious mutations in 1/41 cell lines, from 33 patients (3.0%) is much lower than the population incidence. The reversion mutations and high frequency of heterozygous mutations (14.6%) suggest that there is a selective pressure against BRCA1/2 mutations during adaption to cell culture similar to the selective pressure seen in the clinic after treatment with chemotherapy. PARP inhibitors may be useful in patients with BRCA1 deleterious mutations or gene methylation but further studies of this observation are needed. Some BRCA1/2 wild-type cells are also sensitive to PARP inhibition, particularly those derived from primary tumors. Further research is needed to determine which patients who are BRCA1/2 wild-type will benefit from treatment with PARP inhibitors.

Methods

Cell Culture

Cell lines HOC1, HOC7, HOC8, and IGROV1 were grown in DMEM (Invitrogen, Grand Island, NY, USA # 11995) 10% FBS (Hyclone, Logan, Utah, USA #sv30014.03); OAW42 and CAOV3 were grown in DMEM 10% FBS with the addition of sodium pyruvate (Sigma St. Louis, MO, USA, #S8636), 20ug/mL insulin (Invitrogen #12585-014), L-glutamine (Sigma #G7513) or NEAA (Sigma #M7145)/glucose respectively. FUOV1 was grown in DMEM:F12 (Invitrogen #11330) 10% FBS. OVCA420, OVCA432, OVCA433 were grown in EMEM (ATTC, Manassas, VA, USA, #30-2003) 10% FBS; SW626 was grown in Leibovitz's L15 (ATTC #30.2008) 10% FBS with no CO_2. IOSE29 and IOSE80 were grown in M199:MCDB105 (Invitrogen #11150, Sigma #M6395) 5% FBS. ES2 cells were grown in McCoy's 5a (Invitrogen #116600) 10% FBS with L-glutamine. DOV13 were grown in MEM (Invitrogen #11095) 10% FBS with NEAA. EFO27 were grown in RPMI (ATCC #41458) 20% FBS with the addition of L-glutamine, NEAA and Na Pyruvate; HTB121, was grown in RPMI 20% FBS with the addition of 10μg/ml insulin. The remainder of cells were grown in RPMI-1640 10% FBS, the following cell lines had additives 2mM L-glutamine (HEY, OVCA5, OVCAR3, OVCAR8, A2780, OC316,); 2mM L-glutamine and 10μg/ml insulin (SNU-251and UPN-251). All cell lines were fingerprinted in the MD Anderson CCSG supported cell line characterisation core to establish identity.

DNA Extraction

DNA extractions were performed using the Qiagen QIAamp DNA mini kit "Appendix B: Protocol for Cultured cells" spin column protocol adding 0.4 mg RNaseA to each sample prior to the AL buffer step.

RNA Extraction

All cell lines were grown in Advanced RPMI plus 3% FBS and 1% penicillin/streptomycin 24-48 hours prior to harvesting cells. RNA was extracted using the Qiagen RNeasy mini kit "Animal cells spin" protocol using a QIAshredder for homogenisation and the optional on-column DNase digestion.

BRCA1/2 Analysis

BRCA1/2 mutation screening and qPCR was performed as previously published (Hennessy et al. 2010).

BRCA1 Promoter Methylation qPCR Assays

The Methyl-Profiler DNA Methylation PCR Array System (SA BioSciences) was used to quantify methylation levels following the manufacturers recommended protocol. DNA methylation-sensitive and methylation-dependent restriction enzymes were used to selectively digest unmethylated or methylated genomic DNA, respectively. Post-digest DNA was quantified by real-time PCR using primers flanking the regions of interest, BRCA1 (MePH28472-1A). The relative concentrations of differentially methylated DNA are determined by comparing the amount of each digest with that of a mock digest.

Cytotoxicity-Proliferation Assays

To determine the resistance to chemotherapy drugs, cells were plated into flat-bottomed, 96-well plates at the cell density shown of 1 x 10^3 cells/well and allowed to attach overnight. Slower growing cells (SNU-251) were plated at 2 x 10^3 cells/well. Olaparib (AZD2281) and veliparib (ABT888) were purchased from Selleck Chemicals (Boston, MA, USA) and made up in DMSO. Wells were treated in triplicate with serial dilutions of drug in a final volume of 200μL. Drug-free controls were included in each assay. DMSO controls were also performed for each cell line. Plates were incubated for a further 5 days at 37°C in a humidified atmosphere with 5% CO_2 and cell viability was determined using an acid phosphatase assay (Martin and Clynes 1993). For consistency, all cell lines for cytotoxicity and growth assays were growth in RPMI 10% FCS containing L-glutamine and sodium bicarbonate (Sigma-R8758). Insulin (10μg/ml) was and Sodium pyruvate (1mM) was added to SNU-251. No antibiotics were used in the cell culture. The 2 week SNU-251 cytotoxicity assays followed the same procedure, the media containing drug was changed after 1 week of incubation.

Growth Assays

To determine the growth rate of each cell line 1 x 10^4 cells were plated in 2ml of media in 6-well plates. Duplicate wells were trypsinised and counted every day for 4 days. Data was graphed using Graphpad Prism and doubling time interpolated.

Conflict of Interest

Authors KT, DW, JP, TT, are all employees and shareholders of Myriad Genetics. The remaining authors have no conflict of interests to declare.

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Figure Legends

Figure 1 – Sequence diagrams for BRCA1/2 deleterious mutations.

A) Top: SNU-251 showing homozygous mutation W1815X (5564G>A)

   Bottom: BRCA1 wild-type sequence

B) Top: IGROV-1 showing heterozygous deletion - 2080delA

   Bottom: BRCA1 wild-type sequence

C) Top: UPN-251 showing homozygous deletions - 1199del29 + 1246delA reversion

mutation. Bottom: BRCA1 wild-type sequence

Figure 2 – Quantitative PCR of BRCA1 in ovarian cell line panel. Wild-type cells are

shown with blue circles, methylated cells with green circles and the deleterious mutation

with a red circle.

Figure 3 – Cytotoxicity and doubling time in ovarian cell line panel. Wild-type cells are

shown with blue bars, methylated cells with green bars and the deleterious mutation with

a red bar. A) Cytotoxicity of olaparib in 17 ovarian cancer cell lines. B) Average

cytotoxicity of olaparib by cell and assay type. C) Doubling time of 17 ovarian cancer

cell lines. D) Average doubling time by cell type. E) Cytotoxicity of veliparib in 17

ovarian cancer cell lines. F) Average cytotoxicity of veliparib by cell and assay type.

References

2012a. A2780 ECACC Datasheet. http://www.hpacultures.org.uk .

2012b. CRL-1978™ (ES-2) ATCC Datasheet. http://www.lgcstandards-atcc.org .

2012c. HTB-75™ (CAOV3) ATCC Datasheet. http://www.lgcstandards-atcc.org .

2012d. HTB-78™ (SW626) ATCC Datasheet. http://www.lgcstandards-atcc.org/ .

2000. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Anglian Breast Cancer Study Group. British Journal of Cancer 83 1301-1308.

Aaronson,S.A. 2012. Personal Communication

Alama,A., Barbieri,F., Favre,A. et al. 1996. Establishment and Characterization of Three New Cell Lines Derived from the Ascites of Human Ovarian Carcinomas. Gynecologic Oncology 62 82-88.

Audeh,M.W., Carmichael,J., Penson,R.T. et al. 2010. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. Lancet 376 245-251.

Baldwin,R.L., Nemeth,E., Tran,H. et al. 2000. BRCA1 Promoter Region Hypermethylation in Ovarian Carcinoma: A Population-based Study. Cancer Research 60 5329-5333.

Bauer,K.R., Brown,M., Cress,R.D. et al. 2007. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype. Cancer 109 1721-1728.

Benard,J., Da Silva,J., De Blois,M.C. et al. 1985. Characterization of a Human Ovarian Adenocarcinoma Line, IGROV1, in Tissue Culture and in Nude Mice. Cancer Research 45 4970-4979.

Berchuck,A., Heron,K.A., Carney,M.E. et al. 1998. Frequency of germline and somatic BRCA1 mutations in ovarian cancer. Clinical Cancer Research 4 2433-2437.

Bryant,H.E., Schultz,N., Thomas,H.D. et al. 2005. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 434 913-917.

Buick,R.N., Pullano,R., and Trent,J.M. 1985. Comparative Properties of Five Human Ovarian Adenocarcinoma Cell Lines. Cancer Research 45 3668-3676.

Casalini,P., Botta,L., and Ménard,S. 2001. Role of p53 in HER2-induced Proliferation or Apoptosis. Journal of Biological Chemistry 276 12449-12453.

Choi,J.H., Choi,K.C., Auersperg,N. et al. 2005. Gonadotropins upregulate the epidermal growth factor receptor through activation of mitogen-activated protein kinases and phosphatidyl-inositol-3-kinase in human ovarian surface epithelial cells. Endocrine-Related Cancer 12 407-421.

Colombo,N., Peiretti,M., Castiglione,M. et al. 2009. Non-epithelial ovarian cancer: ESMO Clinical Recommendations for diagnosis, treatment and follow-up. Ann Oncol 20 iv24-iv26.

Colombo,N., Peiretti,M., Parma,G. et al. 2010. Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 21 v23-v30.

Cooke,S.L., Ng,C.K.Y., Melnyk,N. et al. 2010. Genomic analysis of genetic heterogeneity and evolution in high-grade serous ovarian carcinoma. Oncogene 29 4905-4913.

Cruet-Hennequart,S., Maubant,S., Luis,J. et al. 2003. [alpha]v integrins regulate cell proliferation through integrin-linked kinase (ILK) in ovarian cancer cells. Oncogene 22 1688-1702.

DelloRusso,C., Welcsh,P.L., Wang,W. et al. 2007. Functional characterization of a novel BRCA1-null ovarian cancer cell line in response to ionizing radiation. Molecular Cancer Research: MCR. 5 35-45.

Elstrodt,F., Hollestelle,A., Nagel,J.H. et al. 2006. BRCA1 mutation analysis of 41 human breast cancer cell lines reveals three new deleterious mutants. Cancer Research 66 41-45.

Emoto,M., Oshima,K., Ishiguro,M. et al. 1999. Establishment and Characterization of a Serous Papillary Adenocarcinoma Cell Line of the Human Ovary in a Serum-free Culture. Pathology - Research and Practice 195 238-243.

Fajac,A., Da Silva,J., Ahomadegbe,J.C. et al. 1996. Cisplatin-induced apoptosis and p53 gene status in a cisplatin-resistant human ovarian carcinoma cell line. International Journal of Cancer 68 67-74.

Farmer,H., McCabe,N., Lord,C.J. et al. 2005. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434 917-921.

Filmus,J., Trent,J.M., Pullano,R. et al. 1986. A Cell Line from a Human Ovarian Carcinoma with Amplification of the K-ras Gene. Cancer Research 46 5179-5182.

Filmus,J.E. and Buick,R.N. 1985. Stability of c-K-ras Amplification during Progression in a Patient with Adenocarcinoma of the Ovary. Cancer Research 45 4468-4472.

Foray,N., Randrianarison,V., Marot,D. et al. 1999. Gamma-rays-induced death of human cells carrying mutations of BRCA1 or BRCA2. Oncogene 18 7334-7342.

Foulkes,W.D., Metcalfe,K., Sun,P. et al. 2004. Estrogen Receptor Status in BRCA1- and BRCA2-Related Breast Cancer. Clinical Cancer Research 10 2029-2034.

Furlong,M.T., Hough,C.D., Sherman-Baust,C.A. et al. 1999. Evidence for the Colonic Origin of Ovarian Cancer Cell Line SW626. J Natl Cancer Inst 91 1327-1328.

Hamilton,T.C. 2012. Personal Communication.

Hamilton,T.C., Young,R.C., McKoy,W.M. et al. 1983. Characterization of a Human Ovarian Carcinoma Cell Line (NIH:OVCAR-3) with Androgen and Estrogen Receptors. Cancer Research 43 5379-5389.

Helleday,T. 2011. The underlying mechanism for the PARP and BRCA synthetic lethality: Clearing up the misunderstandings. Molecular Oncology 5 387-393.

Hennessy,B.T., Timms,K.M., Carey,M.S. et al. 2010. Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP ribose) polymerase inhibitors in ovarian cancer. Journal of Clinical Oncology 28 3570-3576.

Hills,C.A., Kelland,L.R., Abel,G. et al. 1989. Biological properties of ten human ovarian carcinoma cell lines: calibration in vitro against four platinum complexes. British.Journal of Cancer 59 527-534.

Kao,J., Salari,K., Bocanegra,M. et al. 2009. Molecular Profiling of Breast Cancer Cell Lines Defines Relevant Tumor Models and Provides a Resource for Cancer Gene Discovery. PLoS One 4 e6146.

Kunzmann,R. and Hozel,F. 1987. Karyotype alterations in human ovarian carcinoma cells during long-term cultivation and nude mouse passage. Cancer Genetics and Cytogenetics 28 201-212.

Lakhani,S.R., Manek,S., Penault-Llorca,F. et al. 2004. Pathology of Ovarian Cancers in BRCA1 and BRCA2 Carriers. Clinical Cancer Research 10 2473-2481.

Langdon,S.P., Lawrie,S.S., Hay,F.G. et al. 1988. Characterization and Properties of Nine Human Ovarian Adenocarcinoma Cell Lines. Cancer Research 48 6166-6172.

Li,X., Delzer,J., Voorman,R. et al. 2011. Disposition and Drug-Drug Interaction Potential of Veliparib (ABT-888), a Novel and Potent Inhibitor of Poly(ADP-ribose) Polymerase. Drug Metabolism and Disposition 39 1161-1169.

Liang,Y., McDonnell,S., and Clynes,M. 2002. Examining the relationship between cancer invasion/metastasis and drug resistance. [Review] [134 refs]. Current.Cancer Drug Targets. 2 257-277.

Mackillop,W.J., Trent,J.M., Stewart,S.S. et al. 1983. Tumor Progression Studied by Analysis of Cellular Features of Serial Ascitic Ovarian Carcinoma Tumors. Cancer Research 43 874-878.

Martin,A. and Clynes,M. 1993. Comparison of 5 microplate colorimetric assays for in vitro cytotoxicity testing and cell proliferation assays. Cytotechnology 11 49-58.

Martins,F.C., De,S., Almendro,V. et al. 2012. Evolutionary Pathways in BRCA1-Associated Breast Tumors. Cancer Discovery 2 503-511.

Merkel,D.E., Fuqua,S.A., Tandon,A.K. et al. 1989. Electrophoretic analysis of 248 clinical breast cancer specimens for P-glycoprotein overexpression or gene amplification. J Clin Oncol 7 1129-1136.

National Cancer Institute. 2010. EER Cancer Statistics Review 1975-2007. Lifetime Risk (Percent) of Dying from Cancer by Site and Race/Ethnicity: Males, Total US, 2005-2007 (Table 1.18) and Females, Total US, 2005-2007 (Table 1.19). http://seer.cancer.gov/csr/1975_2007/results_merged/topic_lifetime_risk_death.pdf .

Newman,B., Mu,H., Butler,L.M. et al. 1998. Frequency of Breast Cancer Attributable to BRCA1 in a Population-Based Series of American Women. JAMA 279 915-921.

Nieuwenhuis,B., Assen-Bolt,A.J.V., Van Waarde-Verhagen,W.H. et al. 2002. BRCA1 and BRCA2 heterozygosity and repair of X-ray-induced DNA damage. Int J Radiat Biol 78 285-295.

Pal,T., Permuth-Wey,J., Betts,J.A. et al. 2005. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer 104 2807-2816.

Papelard,H., De Bock,G.H., van Eijk,R. et al. 2000. Prevalence of BRCA1 in a hospital-based population of Dutch breast cancer patients. British Journal of Cancer 83 719-724.

Pohl,G., Ho,C.L., Kurman,R.J. et al. 2005. Inactivation of the Mitogen-Activated Protein Kinase Pathway as a Potential Target-Based Therapy in Ovarian Serous Tumors with KRAS or BRAF Mutations. Cancer Research 65 1994-2000.

Risch,H.A., McLaughlin,J.R., Cole,D.E. et al. 2001. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. American Journal of Human Genetics 68 700-710.

Rottenberg,S., Jaspers,J.E., Kersbergen,A. et al. 2008. High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. Proc Natl Acad Sci U S A 105 17079-17084.

Rouleau,M., Patel,A., Hendzel,M.J. et al. 2010. PARP inhibition: PARP1 and beyond. Nat Rev Cancer 10 293-301.

Rubin,S.C., Blackwood,M.A., Bandera,C. et al. 1998. BRCA1, BRCA2, and hereditary nonpolyposis colorectal cancer gene mutations in an unselected ovarian cancer population: relationship to family history and implications for genetic testing. American Journal of Obstetrics.& Gynecology. 178 670-677.

Russell,P.A., Pharoah,P.D., De Foy,K. et al. 2000. Frequent loss of BRCA1 mRNA and protein expression in sporadic ovarian cancers. International Journal of Cancer 87 317-321.

Sakai,W., Swisher,E.M., Karlan,B.Y. et al. 2008. Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers.[see comment]. Nature 451 1116-1120.

Sakai,W., Swisher,E.M., Jacquemont,C. et al. 2009. Functional Restoration of BRCA2 Protein by Secondary BRCA2 Mutations in BRCA2-Mutated Ovarian Carcinoma. Cancer Research 69 6381-6386.

Schilder,R.J., Hall,L., Monks,A. et al. 1990. Metallothionein gene expression and resistance to cisplatin in human ovarian cancer. International Journal of Cancer 45 416-422.

Sikic,B.I. 2012. Personal Communication.

Sood,A.K., Fletcher,M.S., Gruman,L.M. et al. 2002. The Paradoxical Expression of Maspin in Ovarian Carcinoma. Clinical Cancer Research 8 2924-2932.

Stronach,E.A., Alfraidi,A., Rama,N. et al. 2011. HDAC4-Regulated STAT1 Activation Mediates Platinum Resistance in Ovarian Cancer. Cancer Research 71 4412-4422.

Swisher,E.M., Sakai,W., Karlan,B.Y. et al. 2008. Secondary BRCA1 mutations in BRCA1-mutated ovarian carcinomas with platinum resistance. Cancer Research 68 2581-2586.

Syed,V., Ulinski,G., Mok,S.C. et al. 2001. Expression of Gonadotropin Receptor and Growth Responses to Key Reproductive Hormones in Normal and Malignant Human Ovarian Surface Epithelial Cells. Cancer Research 61 6768-6776.

Timms,K.M. 2011. Personal Communication.

Tung,N., Miron,A., Schnitt,S. et al. 2010a. Prevalence and predictors of loss of wild type BRCA1 in estrogen receptor positive and negative BRCA1-associated breast cancers. Breast Cancer Research 12 R95.

Tung,N., Wang,Y., Collins,L. et al. 2010b. Estrogen receptor positive breast cancers in BRCA1 mutation carriers: clinical risk factors and pathologic features. Breast Cancer Research 12 R12.

Tutt,A., Robson,M., Garber,J.E. et al. 2010. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. The Lancet 376 235-244.

Warren,M., Lord,C.J., Masabanda,J. et al. 2003. Phenotypic effects of heterozygosity for a BRCA2 mutation. Human Molecular Genetics 12 2645-2656.

Wong,A.S.T., Roskelley,C.D., Pelech,S. et al. 2004. Progressive changes in Met-dependent signaling in a human ovarian surface epithelial model of malignant transformation. Experimental Cell Research 299 248-256.

Wong,W.S.F., Wong,Y.F., Ng,Y.T.A. et al. 1990. Establishment and characterization of a new human cell line derived from ovarian clear cell carcinoma. Gynecologic Oncology 38 37-45.

Young,T.N., Pizzo,S.V., and Stack,M.S. 1995. A Plasma Membrane-associated Component of Ovarian Adenocarcinoma Cells Enhances the Catalytic Efficiency of Matrix Metalloproteinase-2. Journal of Biological Chemistry 270 999-1002.

Yuan,Y., Kim,W.H., Han,H.S. et al. 1997. Establishment and characterization of human ovarian carcinoma cell lines. Gynecologic.Oncology 66 378-387.

Yu,J., Roy,D., Brockmeyer,A.D. et al. 2007. Increased chromosomal stability in cultures of ovarian tumours of low malignant potential compared to cystadenomas. British Journal of Cancer 96 1908-1913.

Table 1 – Clinical characteristics of ovarian tumors from which cell lines were established.

| Cell Line | Original Tumor Histology | Isolated From | Pre-Isolation | | Post-Isolation | | References |
			Treatment Received	Response	Treatment Received	Response	
59M	Endometriod/Clear Cell	Ascites	None	N/A	IFO, MEL	PR, Died	(Hills et al. 1989)
A1847	Undifferentiated carcinoma	Metastasis	Unknown	Unknown	Unknown	Unknown	(Aaronson 2012)
A2780	Unknown	Primary Tumour	None	N/A	Unknown	Unknown	(2012a)
CAOV3	Adenocarcinoma	Unknown	Unknown	Unknown	Unknown	Unknown	(2012c)
DOV13	Adenocarcinoma	Unknown	Unknown	Unknown	Unknown	Unknown	(Young et al. 1995)
EFO27	Mucinous	Solid Metastasis	None	N/A	Unknown	Unknown	(Kunzmann and Hozel 1987)
ES2	Serous/Clear Cell	Primary Tumour	None	N/A	Chemotherapy	Refractory	(2012b) (Sikic 2012)
FUOV1	Serous	Primary Tumour	None	N/A	CIS, ASR, CYC	Responded	(Emoto et al. 1999)
HEY	Serous	Peritoneal Deposit and Xenograft	Radiotherapy, Radium	CR	CIS, ADR, CHL	Died	(Buick et al. 1985; Hills et al. 1989)
HI0180	Normal OSE	Normal OSE	N/A	N/A	N/A	N/A	(Sood et al. 2002)
HOC1	Serous	Ascites	MEL, CIS, ADR, CYC	PR, PR	None	N/A	(Buick et al. 1985; Mackillop et al. 1983)
HOC7~	Serous	Ascites	MEL, CIS, ADR, CYC	PR, PR	Unknown	Unknown	(Buick et al. 1985; Mackillop et al. 1983)
HOC8	Serous	Ascites	MEL	PR			(Filmus et al. 1986; Filmus and Buick 1985)
IGROV1	Endometriod/Clear Cell	Primary Tumour	None	N/A	Unknown	Unknown	(Benard et al. 1985)
IOSE29	Normal OSE	Normal OSE + SV40	N/A	N/A	N/A	N/A	(Wong et al. 2004)
IOSE80	Normal OSE	Normal OSE + SV40	N/A	N/A	N/A	N/A	(Choi et al. 2005)
ML46	Serous	Primary Tumour + SV40	Unknown	Unknown	Unknown	Unknown	(Yu et al. 2007)
MPSCI	Serous	Unknown	Unknown	Unknown	Unknown	Unknown	(Pohl et al. 2005)
OAW28	Adenocarcinoma	Ascites	CIS, MEL	NR, NR	Unknown	Died	(Hills et al. 1989)
OAW42	Serous	Ascites	CIS	CR	Unknown	PD, Died	(Hills et al. 1989)
OC316	Serous	Ascites	CIS, ETO, CYC, TAX	PD, SD	Epirubicin	PD	(Alama et al. 1996)
OCC1	Clear Cell	Ascites	Unknown	Unknown	Unknown	Unknown	(Wong et al. 1990)
OVCA420	Serous	Unknown	Unknown	Unknown	Unknown	Unknown	(Syed et al. 2001)

OVCA432	Serous	Unknown	Unknown	Unknown	Unknown	Unknown	(Syed et al. 2001)
OVCA433	Serous	Unknown	Unknown	Unknown	Unknown	Unknown	(Syed et al. 2001)
OVCAR5	Adenocarcinoma	Unknown	None	N/A	Unknown	Unknown	(Schilder et al. 1990)
OVCAR3	Serous	Ascites	CYC, CIS, DOX	Unknown	Unknown	Unknown	(Hamilton et al. 1983; Hills et al. 1989; Schilder et al. 1990)
OVCAR8	Adenocarcinoma	Unknown	CAR	PD	Unknown	Unknown	(Schilder et al. 1990)
OVCAR10	Adenocarcinoma	Unknown	CIS, CAR	PD	Unknown	Unknown	(Schilder et al. 1990)
PA1	Germ Cell Tumor	Ascites	Chemotherapy	NR	Unknown	Died	(Hills et al. 1989)
PEA1	Adenocarcinoma	Pleural Effusion	None	N/A	CIS, PDM	Relapsed	(Langdon et al. 1988)
PEA2*	Adenocarcinoma	Ascites	CIS, PDM	Relapsed	Unknown	Unknown	(Langdon et al. 1988)
PEO1	Serous	Ascites	CIS, CHL, 5-FU	CR	CIS, CHL, 5-FU	CR	(Langdon et al. 1988; Sakai et al. 2009)
PEO4#	Serous	Ascites	CIS, CHL, 5-FU	CR	Higher dose CIS	PD	(Langdon et al. 1988; Sakai et al. 2009)
PEO6#	Serous	Ascites	CIS, CHL, 5-FU, CIS	CR, PD	None	Died	(Langdon et al. 1988; Sakai et al. 2009)
PEO14	Serous	Ascites	None	N/A	CIS, CHL	Relapsed	(Langdon et al. 1988)
PEO23@	Serous	Ascites	CIS, CHL	Relapsed	Unknown	Unknown	(Langdon et al. 1988)
SKOV3	Adenocarcinoma	Ascites	THI	Unknown	Unknown	Unknown	(Hills et al. 1989)
SNU251	Endometriod	Ascites	CYC, ADR, CIS	Unknown	Unknown	Unknown	(Yuan et al. 1997)
SW626	Adenocarcinoma	Primary Tumor or Ovarian Metastasis	Unknown	Unknown	Unknown	Unknown	(2012d; Furlong et al. 1999)
UPN251	Unknown	Unknown	Pt/TAX, TAX	NR	Unknown	Unknown	(Hamilton 2012)

ADR – Adriamycin, CAR- Carboplatin, CIS – Cisplatin, CHL – Chlorambucil, CR – Complete Response, CYC – Cyclophosphamide, DOX – Doxurubicin, ETO – Etoposide, MEL – Melphalan, N/A – Not Applicable, NR – No Response, OSE – Ovarian Surface Epithelium, PD – Progressive Disese, PR – Partial Response, PDM – Prednimustine, Pt – Platinum, TAX – Taxol, THI – Thiotepa. ~Same patient as HOC-1, *Same patient as PEA1, #Same patient as PEO1, @Same patient as PEO14.

Table 2 – BRCA1 sequence variants among ovarian cell lines

Variant	Nucleotide change	Exon	Predicted protein effect	Type of variant	Number mutations in Ovarian Cell lines	No. in BIC
1	1186A>G	11	Q356R	Poly	1	82
2	1199del29 (ctcagagaatcctagagatactgaagatg)	11	Frameshift and then premature stop at base 1218.	Deleterious	1	0
3	1246delA	11	Stop 393	Deleterious	1	4
4	2080delA	11	Stop 700	Deleterious	1	31
5	2196G>A	11	D693N	Poly	7	16
6	2201C>T	11	S694S	Poly	22	14
7	2430T>C	11	L771L	Poly	21	25
8	2577A>G	11	K820E	Poly	1	28
9	2731C>T	11	P871L	Poly	24	30
10	2921G>A	11	Q934Q	Poly	1	0
11	2925G>A	11	D936N	Unclassified	1	0
12	3232A>G	11	E1038G	Poly	20	37
13	3238G>A	11	S1040N	Poly	1	42
14	3667A>G	11	K1183R	Poly	22	32
15	4427T>C	13	S1436S	Poly	21	35
16	4956A>G	16	S1613G	Poly	22	40
17	IVS21+1G>C (First base after exon 21, ie intron 22)	21	Splice Variant	Suspected Deleterious	1	0
18	5564G>A	23	W1815X	Deleterious	1	0

Table 3 – BRCA1/2 mutation, methylation and LOH analysis of ovarian cell lines

Cell Line	LOH	Gene variants	Mutation status	Methylated	LOH	Gene variants	Mutation status
		BRCA1				**BRCA2**	
59M	Yes	None		No	No	5,8,11,25	
A1847	Yes	5,6,7,9,12,14,15,16		Yes	Yes	5	
A2780	No	9		No	No	2,9,11,20,25	
CAOV3	ND	None		No	ND	1,10,23	
DOV13	No	6,7,12,14,15,16		No	No	11,25,26,33	
EFO27	No	9		No	No	1,10,14,21,23	
ES2	ND	5,6,7,9,12,14,15,16		No	ND	11,25,33	
FUOV1	Yes	None		No	No	11,25,33	
HEY	No	1,6,7,9,12,14,15,16		No	No	1,10,23	
HI0180	ND	6,7,9,14,15,16,17*		No	ND	1,10,23	
HOC1	No	6,7,9,12,14,15,16		No	No	1,5,10,23	
HOC7	No	6,7,9,12,14,15,16		No	No	1,5,10,23	
HOC8	ND	6,7,9,12,14,15,16	Wild-type	No	ND	10,23	Wild-type
IGROV1	No	4*,6,7,9,12,14,15,16		No	No	1,10,13,23	
IOSE29	No	6,7,9,12,14,15,16		No	No	5,25	
IOSE80	No	6,7,9,12,14,15,16		No	No	1,23,31*	
ML46	No	None		No	No	None	
MPSCI	No	6,8,9,14,16		No	No	10,11,23,25,32	
OAW28	ND	6,7,9,12,14,15,16		No	ND	1,10,23	
OAW42	No	None		No	No	5,11,25	
OC316	No	5,6,7,9,12,14,15,16		No	No	5,18,19*,25	
OCC1	No	None		No	No	11,25	
OVCA420	No	9		No	No	10,11,22,23,25,33	
OVCA432	Yes	9		No	No	5,25,28,30*	
OVCA433	Yes	None		No	No	5,25,28,30*	
OVCAR5	No	5,6,7,9,12,14,15,16		No	No	5,11,25	

OVCAR3	Yes	None		No	Yes	1,10,23
OVCAR8	Yes	5,6,7,12,14,15,16,		Yes	Yes	None
OVCAR10	ND	9,10,11		No	ND	3,9,11,20,24,25,27
PA1	No	13		No	No	5,12,25
PEA1	Yes	None		No	Yes	1,10,23
PEA2	Yes	None		No	Yes	1,10,23
PEO1	Yes	6,7,9,12,14,15,16		No	Yes	11,15@,16#,25
PEO4	Yes	6,7,9,12,14,15,16		No	Yes	11,17@,25
PEO6	Yes	6,7,9,12,14,15,16		No	Yes	11,17@,25
PEO14	ND	5,6,7,9,12,14,15,16		No	ND	1,5,10,23,25
PEO23	Yes	5,6,7,9,12,14,15,16		No	No	1,5,10,23,25
SKOV3	No	None		No	No	1,10,21,23
SNU251	ND	18	Mutant	No	ND	5,25,29
SW626	Yes	None	Wild-type	No	Yes	4,6,7,9
UPN251	Yes	2#,3#		No	Yes	5,25

* Heterozygous deleterious mutation. # Homozygous Deleterious mutation compensated for by another reversion mutation
@ Reversion Mutation Numbered gene variants for BRCA1 and BRCA2 refer to Tables 2 and 4 respectively.

Table 4 – BRCA2 sequence variants among ovarian cell lines

Variant	Nucleotide change	Exon	Predicted protein effect	Type of variant	Number mutations in Ovarian Cell lines	No. in BIC
1	5'UTR203G>A	n/a	No effect	Poly	16	12
2	602A>G	4	D125G	Uncertain	1	0
3	IVS5-9insT (in intron 5 upstream of exon 6)	6	No effect	Favour Poly	1	2
4	1093A>C	10	N289H	Poly	1	9
5	1342C>A	10	H372N	Poly	16	9
6	1593A>G	10	S455S	Poly	1	7
7	2457T>C	10	H743H	Poly	1	3
8	3031G>A	11	D935N	Poly	1	114
9	3199A>G	11	N991D	Poly	3	3
10	3624A>G	11	K1132K	Poly	18	0
11	4035T>C	11	V1269V	Poly	14	3
12	4097G>A	11	C1290Y	Poly	1	47
13	4542C>T	11	V1438V	Poly	1	0
14	4680C>T	11	D1484D	Poly	1	0
15	5192A>T	11	Y1655L	Poly	1	0
16	5193C>G	11	Stop 1655	Deleterious	1	21
17	5193C>T	11	Y1655Y	Poly	2	0
18	5427C>T	11	S1733S	Poly	1	2
19	5579delA	11	Stop 1790	Deleterious	1	7
20	5932G>A	11	D1902N	Poly	2	87
21	5972C>T	11	T1915M	Poly	2	0
22	6575A>G	11	H2116R	Poly	1	88

23	7470A>G	14	S2414S	Poly	18	10
24	7829C>T	15	A2534V	Favour Poly	1	5
25	IVS16-14C>T (in intron 16 upstream of exon 17)	17	None	Poly	25	15
26	8688A>C	19	V2820V	Poly	1	0
27	9145C>T	22	R2973C	Favour Poly	1	14
28	IVS24-16T>C (intron 24 upstream of exon 25)	25	None	Poly	3	6
29	IVS26-19G>A (intron 26 upstream of exon 27)	27	None	Poly	1	0
30	10204A>T	27	Stop 3326	Poly	3	293
31	10323delCins11	27	Stop 3369	Poly	1	11
32	10349C>T	27	T3374I	Poly	1	8
33	10462A>G	27	I3412V	Poly	4	110

Table 5 – Response of BRCA1/2 wild-type, unmethylated cells to parp inhibitors based on patient characteristics of original tumors.

	Number of Cell Lines	Mean Olaparib IC50	Fold	T-test	Mean Veliparib IC50	Fold	T-test
Primary Tumour	3	2.44 ± 3.14			24.82 ± 29.06		
Metastatic Disease	6	2.92 ± 1.49	1.19	0.87	59.76 ± 11.12	2.00	**0.03**
Chemotherapy Naïve *	4	2.96 ± 2.76			31.69 ± 27.42		
Chemotherapy Pre-treated*	7	2.91 ± 1.40	0.99	0.98	58.15 ± 17.71	1.83	0.08
Responder to Chemotherapy	4	2.92 ± 1.79			59.00 ± 12.67		
Non-Responder to Chemotherapy	4	2.74 ± 1.60	0.93	0.89	45.53 ± 30.48	0.77	0.45
Serous Histology	6	3.50 ± 3.59			34.91 ± 32.84		
Non-Serous Histology	2	3.03 ± 1.71	0.86	0.80	44.90 ± 20.04	1.28	0.61
BRCA1/2 +/+	15	2.29 ± 1.75			42.00 ± 25.99		
BRCA1/2 +/-	4	3.98 ± 1.87	1.73	0.14	42.30 ± 23.68	1.01	0.96
BRCA1/2 -/- (reverted)	2	3.18 ± 2.22	1.38	0.54	68.71 ± 13.32	1.63	0.20

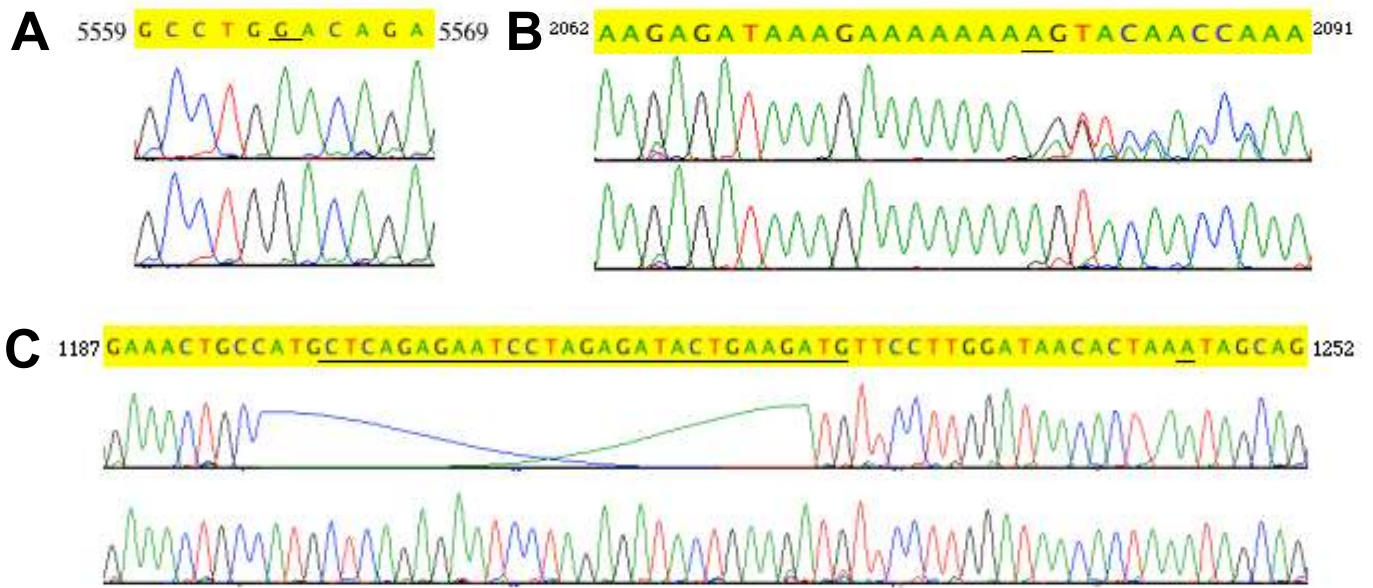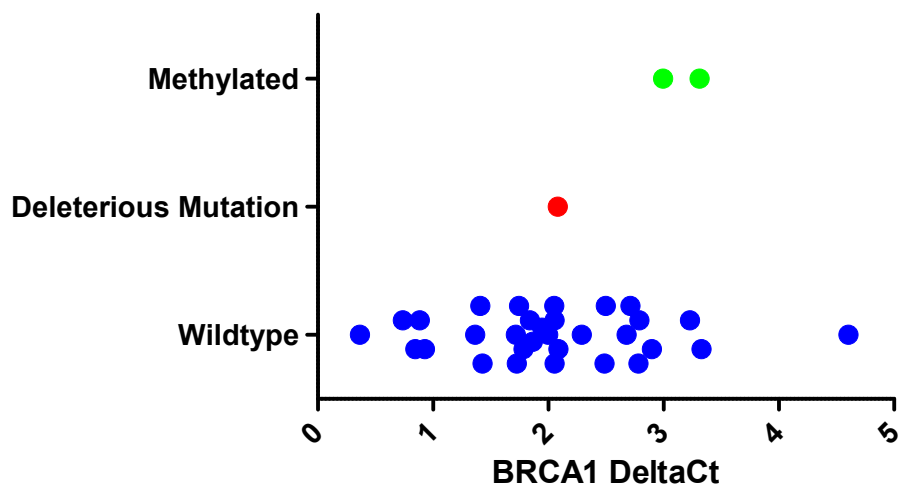*Prior to isolation of the cell line

A 5559 G C C T G G̲ A C A G A 5569   **B** 2062 A A G A G A T A A A G A A A A A A A A G̲ T A C A A C C A A A 2091

C 1187 G A A A C T G C C A T G C T C A G A G A A T C C T A G A G A T A C T G A A G A T G T T C C T T G G A T A A C A C T A A A T A G C A G 1252

Figure 1

Figure 2