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Targeting BRCA1-BER deficient breast cancer by ATM or DNA-PKcs blockade either alone or in combination with cisplatin for personalized therapy

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ABSTRACT

BRCA1, a key factor in homologous recombination (HR) repair may also regulate base excision repair (BER). Targeting BRCA1-BER deficient cells by blockade of ATM and DNA-PKcs could be a promising strategy in breast cancer. We investigated BRCA1, XRCC1 and pol β protein expression in two cohorts ($n = 1602$ sporadic and $n = 50$ germ-line BRCA1 mutated) and mRNA expression in two cohorts ($n = 1952$ and $n = 249$). Artificial neural network analysis for BRCA1-DNA repair interacting genes was conducted in 249 tumours. Pre-clinically, BRCA1 proficient and deficient cells were DNA repair expression profiled and evaluated for synthetic lethality using ATM and DNA-PKcs inhibitors either alone or in combination with cisplatin. In human tumours, BRCA1 negativity was strongly associated with low XRCC1, and low pol β at mRNA and protein levels ($p < 0.0001$). In patients with BRCA1 negative tumours, low XRCC1 or low pol β expression was significantly associated with poor survival in univariate and multivariate analysis compared to high XRCC1 or high pol β expressing BRCA1 negative tumours (ps < 0.05). Pre-clinically, BRCA1 negative cancer cells exhibit low mRNA and low protein expression of XRCC1 and pol β. BRCA1-BER deficient cells were sensitive to ATM and DNA-PKcs inhibitor treatment either alone or in combination with cisplatin and synthetic lethality was evidenced by DNA double strand breaks accumulation, cell cycle arrest and apoptosis. We conclude that XRCC1 and pol β expression status

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in BRCA1 negative tumours may have prognostic significance. BRCA1-BER deficient cells could be targeted by ATM or DNA-PKcs inhibitors for personalized therapy.

1. Introduction

Breast Cancer Susceptibility Gene 1 (BRCA1) facilitates the efficient resolution of DNA double strand breaks (DSBs) through HR (Caestecker and Van de Walle, 2013; Huen et al., 2010). Cells lacking functional BRCA1 protein have impaired HR, and thus depend on the more error-prone non-homologous end joining (NHEJ) pathway leading to chromosomal instability that drive breast cancer development (Huen et al., 2010). In women, BRCA1 germ-line mutation is associated with a 60%–70% lifetime risk of developing breast cancer (O'Donovan and Livingston, 2010). In the more common sporadic breast cancers, epigenetic silencing of the BRCA1 promoter has been reported in up to 11%–14% of tumours (Turner et al., 2004) and a dysfunctional BRCA pathway may also contribute to a BRCAness phenotype in about 25% of cancers (Turner et al., 2004), where breast cancers do not harbour germ-line BRCA mutations but display similar phenotypes including HR deficiency. BER is critical for processing DNA damage caused by alkylation, oxidation, ring saturation, single strand breaks and base deamination. DNA polymerase β (pol β) and XRCC1 are key BER factors. PARP1 (poly [ADP-ribose] polymerase 1) may play an essential role in single strand break repair (SSBR), a BER-related pathway (Langelier and Pascal, 2013). The DNA repair intermediates generated during BER/SSBR, if unrepaired, may get converted to toxic double strand breaks (DSBs) (Dianov and Hubscher, 2013).

Emerging studies suggest a cross talk between BRCA1 and BER factors. BRCA1 mutated and basal-like breast cancer cells were found to be sensitive to oxidative DNA damage induced by H_2O_2 treatment. The increased sensitivity was associated with defective BER as assessed by cell based BER assay in BRCA1 deficient cells (Alli et al., 2009). In a more recent study, BRCA1 deficient cells were sensitive to methyl methane sulfonate (alkylating agent) and functional interaction between pol β and BRCA1 was demonstrated in that study (Masaoka et al., 2013) implying a potential role for pol β in BRCA1 mediated DSB repair. In addition, BRCA1 has also been shown to be involved in the transcriptional regulation of BER factor such as OGG1, NTH1 and APE1 (Saha et al., 2010).

Synthetic lethality is a promising strategy for personalized cancer therapy. PARP [poly-(ADP-ribose) polymerase] inhibitors induce synthetic lethality in germ-line BRCA1 deficient breast cancers and demonstrate clinical benefit in patients (Lord and Ashworth, 2008). Similarly, we have recently shown that APE1 inhibition is synthetically lethal in BRCA1 deficient breast cancer cells and in PTEN (and DSB repair) deficient melanoma cells. The data provides compelling reasons to investigate other potential synthetic lethal interactions targeting DNA repair for clinical application. Cells that are BRCA1 deficient as well as BER impaired may be reliant upon other back-up repair pathways to maintain genomic integrity and survival. ATM and DNA-PKcs play essential roles in the DNA damage response (DDR) and link DNA damage sensing to DDR effectors that regulate cell cycle progression and DNA repair (Shiloh and Ziv, 2013). ATM, a member of the phosphatidylinositol-3-kinase-like protein kinase (PIKK) family, is a key sensor and transducer of DNA damage signalling during HR (Lee and Paull, 2007; Shiloh and Ziv, 2013). ATM recruitment at sites of DNA damage may be dependent upon functional BRCA1 in cells (Lee et al., 2010). DNA-PKcs is another key member of the PIKK family and a critical component of NHEJ pathway required for repair of DSBs generated throughout the cell cycle (Hill and Lee, 2010). BRCA1 through a role in DNA end-processing may also be involved in the regulation of NHEJ (Durant and Nickoloff, 2005).

Our hypothesis is that impaired BER in BRCA1 deficient tumours may influence prognosis. BRCA1-BER deficient cells may be reliant upon ATM or DNA-PKcs mediated back-up pathways for cellular survival and could be targeted by synthetic lethality using inhibitors of ATM or DNA-PKcs.

2. Materials and methods

2.1. Clinical study

2.1.1. BRCA1 and BER protein expression analysis in Nottingham Tenovus Primary Breast Carcinoma cohort

The study was performed in a consecutive series of 1650 patients with primary invasive breast carcinomas who were diagnosed between 1986 and 1999 and entered into the Nottingham Tenovus Primary Breast Carcinoma series described previously (Sultana et al., 2013). Supplemental Table S1 summarizes patient demographics. Supplemental treatment data 1 summarizes various adjuvant treatments received by patients in this cohort.

2.1.2. BRCA1 and BER protein expression analysis in germline BRCA1 deficient breast cancer

The demographics of a cohort of 50 germ-line BRCA1 mutated breast cancers confirmed by genetic testing is shown in Supplementary Table S6. All patients received surgery, adjuvant chemotherapy and radiotherapy according to our institutional policy (supplementary treatment data 1).

2.1.3. Tissue microarrays (TMAs) and immunohistochemistry (IHC)

Tumours were arrayed in tissue microarrays (TMAs) and immunohistochemically profiled for BRCA1, APE1, XRCC1, pol β, and other biological markers (Supplementary Table S2)

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