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Chk1 phosphorylated at serine^{345} is a predictor of early local recurrence and radio-resistance in breast cancer

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ABSTRACT

Radiation-induced DNA damage activates the DNA damage response (DDR). DDR up-regulation may predict radio-resistance and increase the risk of early local recurrence despite radiotherapy in early stage breast cancers. In 1755 early stage breast cancers, DDR signalling [ATM, ATR, total Ckh1, Chk1 phosphorylated at serine^{345} (pChk1), Chk2, p53], base excision repair [PARP1, POLβ, XRCC1, FEN1, SMUG1], non-homologous end joining (Ku70/Ku80, DNA-PKcs) and homologous recombination [RAD51, BRCA1, γH2AX, BLM, WRN, RECQL5, PTEN] protein expression was correlated to time to early local recurrence. Pre-clinically, radio-sensitization by inhibition of Chk1 activation by ATR inhibitor (VE-821) and inhibition of Chk1 (V158411) were investigated in MDA-MB-231 (p53 mutant) and MCF-7 (p53 wild-type) breast cancer cells. In the whole cohort, 208/1755 patients (11.9%) developed local recurrence of which 126 (61%) developed local recurrence within 5 years of initiation of primary therapy. Of the 20 markers tested, only pChk1 and p53 significantly associated with early local recurrence (p value = 0.015 and 0.010, respectively). When analysed together, high cytoplasmic pChk1-nuclear pChk1 (p = 0.039), high cytoplasmic pChk1-p53 (p = 0.004) and high nuclear pChk1-p53 (p = 0.029) co-expression remain significantly linked to early local recurrence. In multivariate analysis, cytoplasmic pChk1 level independently predicted early local recurrence (p = 0.025). In patients who received adjuvant local radiotherapy (n = 949), p53 (p = 0.014) and high cytoplasmic pChk1-p53 (p = 0.017) remain associated with early local recurrence. Pre-clinically, radio-

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sensitisation by VE-821 or V158411 was observed in both MCF-7 and MDA-MB-231 cells and was more pronounced in MCF-7 cells. We conclude that pChk1 is a predictive biomarker of radiotherapy resistance and early local recurrence.

1. Introduction

Despite advances in surgery, adjuvant radiation and systemic therapies, 10–20% of early stage breast cancer patients will develop local recurrence (della Rovere and Benson, 2002; Gieni et al., 2012; Schnitt, 2003; van der Leij et al., 2012). Established risk factors for early local recurrence include young age at diagnosis (≤40 years), larger tumour size, multifocal disease, axillary nodal involvement, extra-capsular tumour extension, positive margin, high grade, definite positive lympho-vascular invasion, HER-2 overexpression, ER negativity, and extensive intra-ductal component (della Rovere and Benson, 2002; Gieni et al., 2012; Schnitt, 2003; van der Leij et al., 2012). Local recurrence may herald the emergence of radio-resistant cancer clones with aggressive biology that may adversely impact upon patient outcomes (Clarke et al., 2005). Therefore, mining for predictive biomarkers for development of radio-resistance is a high priority. Ionizing radiation (IR) induced DNA damage is a key mechanism for cytotoxicity and therapeutic efficacy in tumours (Derks et al., 2014; Santivasi and Xia, 2014). However, the ability of tumour cells to initiate an effective DNA damage response (DDR), by activating multiple DNA damage signalling and repair pathways, may result in resistance to radiotherapy and ultimately influence the emergence of local recurrence in patients (Derks et al., 2014; Santivasi and Xia, 2014).

In the current study, we have comprehensively investigated the expression of a panel of DNA repair factors involved in the DDR: DNA damage signalling (ATM, ATR, total Chk1, Chk1 phosphorylated at serine^{345} (here in referred to as pChk1) (Tian et al., 2015), CHK2, p53, base excision repair (PARP1, POLβ, XRCC1, FEN1, SMUG1) (Wallace, 2014), non-homologous end joining (Ku70/Ku80, DNA-PKcs) (Williams et al., 2014) and homologous recombination (RAD51, BRCA1, γH2AX, BLM, WRN, RECQL5, PTEN) (Liu and Huang, 2014) for local recurrence prediction in early stage breast cancers. In addition, work was also undertaken to test the blockade of CHK1 activation through ATR inhibition (VE-821, Vertex pharmaceuticals) (Josse et al., 2014) and direct CHK1 inhibition (V158411, Vernalis R&D Ltd) (Bryant et al., 2014; Stokes et al., 2009) in MDA-MB-231 and MCF-7 breast cancer cell lines.

2. Methods

2.1. Clinical study

2.1.1. Patients

Demographics of the study population are summarised in Table 1. The cohort comprised of primary operable early-stage (stage I–III) invasive breast cancers from patients treated by breast-conserving surgery (wide local excision) and radiotherapy at Nottingham University Hospitals. Information on clinical history and outcome is prospectively maintained and patients were assessed in a standardised manner for clinical history and tumour characteristics. Local recurrence-free survival was defined as the time interval (in months) between the start of primary treatment and date of first histological confirmation of recurrent cancer (invasive or *in-situ*) at the vicinity of the treated breast. Distant metastasis-free survival was defined as the time interval (in months) between the start of primary treatment and date of distant disease relapse. Breast Cancer Specific Survival (BCSS) is defined as the time (in months) from the date of primary surgery to the date of breast cancer related death.

In this early breast cancer cohort, 949 patients had received adjuvant local radiotherapy. Patients were managed in accordance to a uniform protocol, where all underwent wide local excision followed by radiotherapy which was given in daily 2 Gray (Gy) fractions, to a total dose of 50–55 Gy over 5 weeks. Thirty one patients received radiotherapy in the context of clinical trials investigating altered fractionation schedules. Radiotherapy was delivered to the whole breast. During the treatment period a "boost" to the tumour bed was not routinely given, however a "16 Gy boost" was given to six patients. Patients received systemic adjuvant treatment on the basis of Nottingham Prognostic Index (NPI), estrogen receptor (ER) status and menopausal status. Patients with an NPI score <3.4 did not receive adjuvant treatment. ER negative or pre-menopausal cases with an NPI score of 3.4 or more were candidates for cyclophosphamide, Methotrexate and 5-Flourouracil (CMF) combination chemotherapy; ER positive cases received Tamoxifen for a period of 5 years.

This study is reported according to REMARK (Reporting Recommendations for Tumour Marker Prognostic Studies)

Table 1 – Multivariate analysis (Time to local recurrence).

	P value	Exp(B)	95.0% CI for Exp(B)	
			Lower	Upper
Time to local recurrence				
Lymph node status	0.710	0.920	0.591	1.431
Tumour size	0.592	0.888	0.575	1.371
Tumour Grade	0.396	1.165	0.818	1.659
ER	0.410	0.791	0.453	1.381
Vascular Invasion	0.216	1.488	0.793	2.794
p53	0.315	1.278	0.792	2.062
pChk1 (cytoplasmic)	**0.025**	1.639	1.065	2.522

Bold = statistically significant.

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