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Genetic polymorphisms in DNA repair and damage response genes and late normal tissue complications of radiotherapy for breast cancer

J Chang-Claude*[,1], CB Ambrosone[2], C Lilla[1], S Kropp[1], I Helmbold[1], D von Fournier[3], W Haase[4], M-L Sautter-Bihl[5], F Wenz[6], P Schmezer[7] and O Popanda[3]

[1]Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany; [2]Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, NY, USA; [3]Department of Gynecological Radiology, University Hospital Heidelberg, Heidelberg, Germany; [4]Clinic for Radiotherapy and Radiooncology, St Vincentius-Clinics Karlsruhe, Karlsruhe, Germany; [5]Clinic for Radiotherapy, Municipal Hospital Karlsruhe, Karlsruhe, Germany; [6]Department of Radiation Oncology, University Hospital Mannheim, Mannheim, Germany; [7]Division of Epigenomics and Cancer Risk Factors, German Cancer Research Center, Heidelberg, Germany

Breast-conserving surgery followed by radiotherapy is effective in reducing recurrence; however, telangiectasia and fibrosis can occur as late skin side effects. As radiotherapy acts through producing DNA damage, we investigated whether genetic variation in DNA repair and damage response confers increased susceptibility to develop late normal skin complications. Breast cancer patients who received radiotherapy after breast-conserving surgery were examined for late complications of radiotherapy after a median follow-up time of 51 months. Polymorphisms in genes involved in DNA repair (*APEX1, XRCC1, XRCC2, XRCC3, XPD*) and damage response (*TP53, P21*) were determined. Associations between telangiectasia and genotypes were assessed among 409 patients, using multivariate logistic regression. A total of 131 patients presented with telangiectasia and 28 patients with fibrosis. Patients with variant *TP53* genotypes either for the Arg72Pro or the PIN3 polymorphism were at increased risk of telangiectasia. The odds ratios (OR) were 1.66 (95% confidence interval (CI): 1.02–2.72) for 72Pro carriers and 1.95 (95% CI: 1.13–3.35) for PIN3 A2 allele carriers compared with non-carriers. The *TP53* haplotype containing both variant alleles was associated with almost a two-fold increase in risk (OR 1.97, 95% CI: 1.11–3.52) for telangiectasia. Variants in the *TP53* gene may therefore modify the risk of late skin toxicity after radiotherapy.

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Radiotherapy is commonly applied after breast-conserving surgery to reduce the risk of locoregional recurrence of breast cancer and has been shown to be as effective as radical mastectomy (Fisher *et al*, 2002). Although standard radiation therapy is well tolerated by the majority of patients, late normal tissue complications arising from the intrinsic sensitivity of normal tissue, and correlated poor cosmetic results, remain as health concerns of treated breast cancer patients over time (Cetintas *et al*, 2002; Deutsch and Flickinger, 2003; Smith and Ross, 2004). The process of endothelium reconstruction is radiation dose-dependent, progresses over months and years and leads to increases in the severity of both telangiectasia and fibrosis (Bentzen *et al*, 1989; Archambeau *et al*, 1995; Chen *et al*, 2006). Telangiectasias are small dilated blood vessels near the surface of the skin and fibrosis is the development of excess fibrous connective tissue leading to induration. There is, however, considerable inter-individual variability in the development of adverse reactions in normal tissue of irradiated patients. Besides duration, radiation dose and schedule (Turesson *et al*, 1996; Hill *et al*, 2001), patient-related factors, such as age, acute skin reaction and lifestyle factors (Bentzen and Overgaard, 1991; Bentzen *et al*., 1996; Turesson *et al*, 1996; Johansen *et al*, 2002; Deutsch and Flickinger, 2003; Chen *et al*, 2006; Lilla *et al*, 2007), as well as genetic susceptibility (Bentzen and Overgaard, 1994; Chang-Claude *et al*, 2005; Andreassen *et al*, 2006; Popanda *et al*, 2008) have been implicated. Sensitivity to radiation exposure is suggested to be a complex, polygenic trait, which results from the interaction of a number of genes in different cellular pathways (Travis, 2007).

As radiation therapy exerts its cytotoxic effects through damage to cells, proteins and DNA, the individual capacity to repair damaged DNA may modify the response of the normal tissue. Radiation-induced DNA damage is diverse and therefore nearly all DNA repair pathways might be involved in its removal, especially repair of double-strand breaks through mechanisms such as homologous recombination and non-homologous end joining (Jeggo and Lobrich, 2006). In addition, nucleotide and base-excision repair play an important role, mainly in the repair of oxidative DNA damage (Hoeijmakers, 2001).

*Correspondence: Professor Dr J Chang-Claude, Division of Cancer Epidemiology, C020, German Cancer Research Center, Im Neuenheimer Feld 280, Heidelberg 69120, Germany;
E-mail: j.chang-claude@dkfz.de
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Furthermore, the complex response to ionising radiation requires the expression and activity of the p53 pathway (Gudkov and Komarova, 2003). The p53 protein is activated through phosphorylation by radiation DNA damage-induced kinases, including ataxia telangiectasia-mutated and the DNA-dependent protein kinase (Banin *et al*, 1998; Fei and El-Deiry, 2003; Schwartz, 2007). Activated p53 protein has various downstream targets, including genes involved in cell-cycle regulation, apoptosis, and DNA repair. Regulation of these processes by p53 controls the cellular response to ionising radiation-induced damage. *p21* is a critical cell-cycle checkpoint gene, regulated tightly by p53. As soon as DNA is damaged by radiation, binding of p53 protein induces transcription of the downstream gene *p21*, which stops cells from entering into the S phase (Robles *et al*, 2002). p21, together with p53, is directly involved in G1/S checkpoint control in response to ionising radiation (Dotto, 2000).

We therefore evaluated the association between several putative functional polymorphisms in six genes involved in DNA repair and two damage response genes and development of late normal tissue complications in a prospective study of breast cancer patients who received radiotherapy after breast-conserving surgery.

MATERIAL AND METHODS

Patient population and data collection

The methods of this study have been described earlier (Twardella *et al*, 2003; Chang-Claude *et al*, 2005; Lilla *et al*, 2007). Briefly, women diagnosed with breast cancer who received radiotherapy after breast-conserving surgery were enrolled between June 1998 and March 2001 from four radiotherapy units in Germany (Women's Clinic at the University of Heidelberg, St Vincentius Clinic in Karlsruhe, City Hospital in Karlsruhe and University Hospital of Mannheim). Patients who received chemotherapy before or during radiation were not eligible for the study. Information on demographic factors, medical history, and lifestyle factors was obtained through self-administered questionnaires. Details on clinical tumor characteristics and treatment regimen were abstracted from patient records. Informed consent was obtained from all participants, and the study was approved by the ethics committee of the University of Heidelberg, the Institutional Review Board for Roswell Park Cancer Institute, and the US Army Medical Research and Materiel Command Human Subjects Research Review Board.

Breast irradiation

Details on the radiotherapy regimen (total dose, dose per fraction, treatment time, boost dose) were abstracted from the irradiation protocols. As described earlier (Twardella *et al*, 2003), all patients received a common breast irradiation treatment with conformal tangential irradiation with lateral and medial wedge fields, including CT-based planning, simulation, verification, and quality assurance. At three hospitals, the standard regimen included irradiation of the whole breast, either 50 Gy given in 5×2.0 Gy fractions or 50.4 Gy in 5×1.8 Gy fractions per week, followed by a photon or electron boost with doses ranging from 5 to 20 Gy. Three patients were treated with brachytherapy (20 or 25 Gy). In the fourth radiation department, patients received 56 Gy of whole breast irradiation in 5×2.0 Gy fractions without boost. The biologically effective dose (BED) of radiotherapy relative to an irradiation with a fraction dose of 2.0 Gy, that is the normalised total dose (NTD), was calculated to account for differences in fractionation according to the following formula:

$$\mathrm{NTD} = \frac{\mathrm{BED}}{1 + 2\mathrm{GY}/(\alpha/\beta)} = \mathrm{n.d.}\frac{(1 + d/(\alpha/\beta))}{(1 + 2\mathrm{GY}/(\alpha/\beta))}$$

given the number of fractions n, the fraction size of d, and an α/β ratio of 3 Gy for telangiectasia and 2 Gy for fibrosis.

Follow-up and evaluation of toxicities

The occurrence of acute side effects of radiotherapy was monitored and documented by physicians several times during the study. We have earlier reported on acute radiation-induced toxicity, defined as grade 2c and above (at least one moist desquamation or interruption of radiotherapy due to toxicity), in this patient cohort (Twardella *et al*, 2003; Chang-Claude *et al*, 2005; Ambrosone *et al*, 2006; Popanda *et al*, 2006; Tan *et al*, 2006). Patients were recontacted between June 2003 and July 2005 to assess the occurrence of late adverse effects of radiotherapy and course of disease (relapse, metastases, secondary carcinoma, and death). A self-administered questionnaire similar to that applied at baseline was used to collect information on demographic and epidemiological risk factors, and to record behavior changes that may have occurred after radiotherapy. Patients were examined by the study physician or the treating physician to assess the occurrence of late adverse effects of radiotherapy.

The late side effects were classified according to the RTOG/EORTC late radiation morbidity scoring schema (Seegenschmiedt, 1998) supplemented by LENT-SOMA scores. Patients' general condition, weight changes, nausea and development of lymphatic edema (arm or breast), and adverse reactions of the skin (telangiectasia), subcutaneous tissue (fibrosis) and other organ tissues (heart, lung, larynx) were recorded. The severity of late effects was scored from 0 to 4, whereby the development of side effects of scores $\geqslant 2$ was considered to indicate late normal tissue complications.

Genotyping assays

Most polymorphisms (see Table 2) were detected by amplification with real-time PCR followed by melting-curve analysis with fluorescence-labeled hybridisation probes in a LightCycler (Roche Diagnostics, Mannheim, Germany) as described earlier (Chang-Claude *et al*, 2005; Popanda *et al*, 2006; Tan *et al*, 2006). The oligonucleotides for analysis of the *XRCC1* -77 polymorphism (rs3213245) were the PCR primers (sense) 5′-ctttagccagcgcaggtcg-3′OH and (antisense) 5′-ccccatgcaggtccctcac-3′OH, sensor 5′-cccgccccctcccactc-3′-FL and anchor 5′-LC Red640-ccctgcccct cggaccccatactc-3′P. The sense primer included a mismatch to avoid stem loops in the amplicon because of the high and repetitive G/C content of the target sequence. PCR primers and probes were designed with the help of Tib Molbiol (Berlin, Germany). Annealing temperature of the primers was 60°C. The PCR was performed for all polymorphisms with Qiagen reagents (Qiagen, Hilden, Germany) in a volume of 10 μl using 10 ng of DNA. Overall, 10% randomly selected samples were analysed by conventional PCR-RFLP to verify the LightCycler results; 100% concordance was found. The insertion of the *TP53* PIN3 polymorphism was identified by standard PCR and electrophoresis (Tan *et al*, 2006). A negative control containing all the reagents but with water instead of the DNA template was included in every amplification set. All genotyping assays were carried out blinded to the clinical diagnosis. For each polymorphism, PCR fragments of the homozygous wild-type allele, the homozygous variant allele, and one heterozygous sample were sequenced.

Statistical analysis

Significant differences in distribution of genotypes by presence of late skin toxicities (scores $\geqslant 2$) were tested by the χ^2 and Fischer's exact tests. Each polymorphism was tested for deviation from Hardy–Weinberg equilibrium by comparing the observed and expected genotype frequencies using the χ^2-test with one degree of freedom. Multivariate unconditional logistic regression analysis was used to assess the association of genotypes with occurrence of late complications of radiotherapy. Odds ratios (OR) and 95%

confidence intervals (CI) were computed using the LOGISTIC procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Possible effect modification of genotype associations by other covariables was evaluated by the log likelihood ratio test comparing models with and without the first-order interaction terms. All tests were two-sided and considered to be statistically significant with a *P*-value of $\leqslant 0.05$. The logistic regression analysis was performed only for the occurrence of telangiectasia, excluding seven patients who developed fibrosis with a score $\geqslant 2$ but not telangiectasia. Multivariate models included NTD, age at the time of late toxicity evaluation, and follow-up time since end of radiotherapy. Stepwise backward elimination with $P \leqslant 0.03$ as threshold was used to develop a final model to control for potential confounders. The final model included, in addition hospital facility, acute skin toxicity, a history of hypertension and allergy, skin type (three categories), pack-years of smoking (0, 1–19, $\geqslant 20$), and marital status.

Analyses to assess association between haplotypes and risk for telangiectasia were carried out using the function haplo.glm of the R package haplo.stats, which uses a generalised linear model (glm) allowing for an ambiguous linkage phase (Lake *et al*, 2003). The most common haplotype was used as the referent. Possible effect modification of haplotype associations by other covariables were evaluated by the likelihood ratio test. As this was a hypothesis generating study, significance level was defined at $P < 0.05$ although 13 SNPs were tested.

Results

Data on late effects of radiotherapy as well as information on demographic and epidemiological factors were available for 421 breast cancer patients, as reported earlier (Lilla *et al*, 2007; Kuptsova *et al*, 2008). After a median follow-up time of 51 months (range 36–77 months), the most common symptoms of scores $\geqslant 2$, which were observed included telangiectasia (32.1%), impairment of the general condition (15.9%), fibrosis (7.1%), lymphatic edema in the arm and breast (6.2%), and pain (5.5%). Of 416 patients (after excluding 3 patients treated with interstitial boost and 2 patients with missing information on fibrosis), 131 patients presented with telangiectasia and 28 with fibrosis of grades $\geqslant 2$, whereby 21 patients presented with both adverse reactions. Characteristics of the 409 breast cancer patients who also had genotype data and were included in this analysis (excluding the seven patients presenting with fibrosis only) are shown in Table 1.

We found a significant association between genetic polymorphisms in the *TP53* gene and risk for telangiectasia (Table 2). Compared with non-carriers, patients carrying the variant *TP53* 72Pro allele had an increased risk of adverse effects (OR of 1.66, 95% CI: 1.02–2.72). Carriers of the *TP53* PIN3 A2 allele were also at increased risk of telangiectasia (OR 1.95, 95% CI: 1.13–3.35). None of the other genetic polymorphisms studied showed significant associations with occurrence of telangiectasia.

Strong association (linkage disequilibrium) was found between the *TP53* Arg72Pro and *TP53* PIN3 polymorphisms ($P < 0.001$). We therefore investigated haplotype effects of the two *TP53* polymorphisms. Compared with the common ArgA1 haplotype, the ProA2 haplotype containing both variant alleles was associated with a significantly increased OR of 1.97 (95% CI: 1.11–3.52) for telangiectasia (Table 3). Haplotype association analysis for the *XRCC1* and *XPD* genes with data for at least two genetic polymorphisms did not reveal further significant findings.

Further analysis for effect modification yielded differences in the effect of *TP53* on risk for telangiectasia, according to occurrence of acute skin toxicity (moist desquamation). Thirty women (22.9%) had presented with acute skin toxicity during radiotherapy in patients with telangiectasia, and 45 women (16.2%) in those without telangiectasia. The elevated risk of telangiectasia associated with the *TP53* ProA2 haplotype was found only in patients who did not present with acute toxicity during radiotherapy (OR 2.78, 95% CI: 1.44–5.35) and not in those who experienced acute skin toxicity during radiotherapy ($P_{\text{heterogeneity}} = 0.06$) (Table 3).

Table 1 Clinical and demographic characteristics of the breast cancer patients

Characteristics	Mean (s.d.)	Range
Age late toxicities (years)[a]	60.6 (8.57)	27–88
Age radiotherapy (years)[b]	64.7 (8.59)	31–91
Total radiation dose (Gy)[c]	61.8 (4.10)	51–71
Follow-up time (months)	51.4 (6.81)	36–77
	Frequency	**Percent**
Body mass index (kg/m^2)		
<25	182	44.5
25–30	161	39.4
>30	66	16.1
Tumor stage status		
In situ	36	8.8
1	277	67.7
2	92	22.5
Other or unknown	1	0.2
Lymph node status		
0	314	76.8
1	57	13.9
Unknown	38	9.3
Metastasis status		
0	261	63.8
1	1	0.2
Unknown	147	35.9
Boost therapy type		
Photon	275	67.2
Electron	94	23.0
No boost	40	9.8
Radiotherapy clinic		
University of Heidelberg Women's Clinic	228	55.8
Karlsruhe St Vincentius clinic	96	23.5
Karlsruhe City Hospital	60	14.7
University Hospital of Mannheim	25	6.1

[a]Age at the time of late toxicities evaluation. [b]Age at the end of radiation therapy. [c]Includes irradiation to the whole breast and boost application.

DISCUSSION

In this study of breast cancer patients treated with radiotherapy after breast-conserving surgery, we found that variants of *TP53* were associated with an increased risk for developing telangiectasia after radiation therapy. Although both variants, TP53 72Pro and PIN3 A2, were associated with elevated risk, the haplotype results suggested that *cis* effects of the two variants may be most relevant.

Two of the many p53 functions may be important in modulating radiosensitivity. Growth arrest mediated by p53 plays an important role in inhibiting mitotic cell death in epithelia of the small intestine of mice and, thus, is thought to reduce radiation toxicity in these animals (Komarova *et al*, 2004). Also, apoptosis and cell death by mitotic catastrophe have been recognised as an important response to radiation in many cells (Dewey *et al*, 1995; Weber and Wenz, 2002; Komarova *et al*, 2004) as they remove heavily

Table 2 Association between polymorphisms in DNA repair and cell-cycle genes and risk of developing late skin toxicity (telangiectasia) with score ⩾2 after radiotherapy

		Patients without telangiectasia		Patients with telangiectasia			
Gene polymorphism	**Genotype**	***N*=278**	**%**	***N*=131**	**%**	**OR[a]**	**95% CI**
APEX1	TT	71	25.9	39	30.7	1.00	
Asp148Gln	TG	134	48.9	65	51.2	1.03	0.58–1.83
rs3136820	GG	69	25.2	23	18.1	0.66	0.33–1.32
	TG+GG	203	74.1	88	69.3	0.90	0.53–1.54
XRCC1	TT	94	34.2	43	33.9	1.00	
−77 T>C	TC	136	49.5	54	42.5	0.97	0.56–1.67
rs3213245	CC	45	16.4	30	23.6	1.87	0.94–3.70
	TC+CC	181	65.8	84	66.1	1.17	0.71–1.95
XRCC1	CC	242	88.3	117	92.1	1.00	
Arg194Trp	CT	30	11	10	7.9	0.58	0.24–1.40
rs1799782	TT	2	0.7	0	0	0	0
	CT+TT	32	11.7	10	8	0.57	0.24–1.38
XRCC1	GG	244	88.4	118	92.9	1.00	
Arg280His	GA	30	10.9	9	7.1	0.49	0.19–1.24
rs25489	AA	2	0.7	0	0	0	0
	GA+AA	32	11.6	9	7.1	0.43	0.17–1.09
XRCC1	GG	112	40.6	50	39.4	1.00	
Arg399Gln	GA	120	43.5	63	49.6	1.09	0.65–1.82
rs25487	AA	44	15.9	14	11	0.63	0.29–1.37
	GA+AA	164	59.4	77	60.6	0.96	0.59–1.57
XRCC2	GG	236	85.5	113	89	1.00	
Arg188His	GA	38	13.8	13	10.2	0.83	0.39–1.76
rs3218536	AA	2	0.7	1	0.8	1.05	0.08–13.93
	GA+AA	40	14.5	14	11	0.84	0.41–1.74
XRCC3	CC	104	38	45	35.4	1.00	
Thr241Met	CT	126	46	63	49.6	1.05	0.62–1.79
rs861539	TT	44	16.1	19	15	1.12	0.53–2.40
	CT+TT	170	62	82	64.6	1.07	0.65–1.77
NBS1	GG	120	43.5	53	41.7	1.00	
Glu185Gln	GC	137	49.6	58	45.7	0.92	0.55–1.54
rs1805794	CC	19	6.9	16	12.6	2.14	0.88–5.19
	GC+CC	156	56.5	74	58.3	1.06	0.65–1.72
XPD	GG	120	43.8	42	33.3	1.00	
Asp312Asn	GA	117	42.7	69	54.8	1.51	0.89–2.55
rs1799793	AA	37	13.5	15	11.9	0.91	0.41–2.01
	GA+AA	154	56.2	84	66.6	1.36	0.82–2.24
XPD	AA	109	39.6	42	33.3	1.00	
Lys751/Gln	AC	133	48.4	65	51.6	1.15	0.68–1.95
rs13181	CC	33	12	19	15.1	1.21	0.57–2.58
	AC+CC	166	60.4	84	66.6	1.16	0.70–1.92
P21	CC	242	87.7	110	86.6	1.00	
Ser31Arg	CA	31	11.2	17	13.4	1.54	0.71–3.32
rs1801270	AA	3	1.1	0	0	0	0
	CA+AA	34	12.3	17	13.4	1.27	0.60–2.68
TP53	GG	160	58.0	64	50.4	1.00	
Arg72Pro	GC	96	34.8	49	38.6	1.67	0.98–2.83
rs1042522	CC	20	7.3	14	11	1.62	0.71–3.70
	GC+CC	116	40	63	49.6	1.66	1.02–2.71
TP53	A1A1	214	77.6	87	68.5	1.00	
PIN3	A1A2	56	20.3	40	31.5	2.14	1.23–3.71
	A2A2	6	2.2	0	0	0	0
	A1A2+A2A2	62	22.4	40	31.5	1.95	1.13–3.37

CI = confidence interval; NTD = normalised total dose; OR = odds ratio. [a]Adjusted for NTD, age at the time of late toxicities evaluation, time since radiotherapy (months), clinic, acute skin toxicity, high blood pressure, allergy, pack-years (never, <20, ⩾20), skin type (always/moderate/seldom sunburn), clinic, marital status (single/divorced/widowed, married/partner).

Table 3 Reconstructed haplotypes and the association with risk of developing late skin toxicity (telangiectasia) with score ⩾2 after radiotherapy

Gene	Haplotype	Frequency	OR[a]	95% CI
All patients				
TP53	GA1	0.71	1	
	GA2	0.03	1.02	0.29–3.63
	CA1	0.16	1.20	0.79–1.82
	CA2[b]	0.11	1.97	1.11–3.52
Patients without acute toxicity during radiotherapy[c]				
TP53	GA1	0.71	1	
	GA2	0.02	0.68	0.13–3.60
	CA1	0.16	1.15	0.71–1.85
	CA2	0.12	2.78	1.44–5.37
Patients with acute toxicity during radiotherapy				
TP53	GA1	0.71	1	
	GA2	0.04	1.10	0.09–13.41
	CA1	0.17	1.47	0.52–4.17
	CA2	0.09	0.52	0.11–2.53
All patients				
XRCC1	CCGG	0.41	1	
	TCGG	0.12	1.15	0.66–2.00
	TCGA	0.36	0.78	0.53–1.15
	TTGG	0.05	0.51	0.21–1.24
	Rare[d]	0.07	0.56	0.26–1.20
XPD	GA	0.56	1	
	GC	0.09	1.25	0.64–2.46
	AA	0.06	1.14	0.55–2.38
	AC	0.29	1.09	0.74–1.62

CI = confidence interval; NTD = normalised total dose; OR = odds ratio. [a]Adjusted for NTD, age the time of late toxicities evaluation, time since radiotherapy (months), clinic, acute skin toxicity, high blood pressure, allergy, pack-years (never, <20, ⩾20), skin type (always/moderate/seldom sunburn), clinic, marital status (single/divorced/widowed, married/partner). [b]A2 allele carries a duplication of 16 bp in intron 3. [c]$P = 0.06$ for effect heterogeneity according to occurrence of acute skin toxicity. [d]Composed of haplotypes with frequencies below 5%.

damaged cells from the tissue. Functional analysis of the two *TP53* variants in codon 72 showed that this polymorphism might modulate these two responses. The 72Arg form induced apoptosis more efficiently than the 72Pro form. In contrast, the 72Pro form appeared to induce a higher level of G1 arrest than the 72Arg form giving time to repair (Thomas *et al*, 1999; Dumont *et al*, 2003; Pim and Banks, 2004). Consequently, the 72Pro p53 protein was found to be more efficient in specifically activating p53-dependent DNA repair target genes, and cells carrying the 72Pro allele had significantly higher DNA repair capacity (Siddique and Sabapathy, 2006). Although it is unclear which of the functional differences between the codon 72 polymorphic alleles is more important, our results could be explained by the lower efficiency with which the 72Pro form induced apoptosis of heavily damaged cells after radiation. Repair and reconstitution of the normal tissue function might be incomplete over time in the presence of these cells, leading to late adverse effects which become visible as telangiectasia, a disturbance of the blood vessels.

We also observed an independent effect of the *TP53* PIN3 polymorphism on the risk of late radiation toxicity, but the results of the haplotype analysis suggested the strongest effect on risk conferred by the haplotype containing both variant alleles. The functional significance of *TP53* PIN3 has remained largely unexplored. Our haplotype analysis revealed further that the strongest risk effect of the 72ProA2 haplotype was visible in patients who did not develop severe acute side effects during radiotherapy. We proposed that the Pro allele carriers experienced reduced cell loss by apoptosis and, potentially, mitotic catastrophe during therapy and were, thus, protected from severe acute side effects as we found in our analysis of acute side effects (Tan *et al*, 2006). This protective effect may turn out as a risk factor for late side effects when the irradiated tissue is observed over a longer time. More analyses of the *in vivo* and *in vitro* effects of the *TP53* Arg72Pro and PIN3 polymorphisms are needed, however, before we can apply these *TP53* variants as predictive markers for late side effects of radiotherapy.

p21 plays a direct role in mediating irradiation-induced G1 arrest, with p53 as the transcription factor in this process. This mechanism indicates a possible combined effect of polymorphisms in the two genes. However, p53 may modulate response to radiation damage in the G1 phase of the cell cycle through mechanisms independent of p53-mediated transcriptional activation of p21 and cell-cycle arrest (Mazzatti *et al*, 2005). We did not observe a significant effect of *p21* Ser31Arg polymorphism on the risk of late skin toxicity. Other studies also failed to find an association of this variant with risk or prognosis of breast cancer (Keshava *et al*, 2002; Azzato *et al*, 2008).

In addition, ten polymorphisms causing an amino acid change in six different DNA repair genes were investigated for associations with telangiectasia, but no significant effects were detected. The *XRCC1* Arg399Gln polymorphism has been reported to be associated with telangiectasia but not with fibrosis, particularly in patients who did not receive a boost, albeit based on 167 patients of whom 39 presented with telangiectasia (Giotopoulos *et al*, 2007). This polymorphism was also not found to be associated with severe grade 3 fibrosis after irradiation of the breast (Andreassen *et al*, 2006). A further study, which did not differentiate between early and late adverse reaction to radiotherapy, reported an elevated risk in women carrying both the variant alleles of the Arg194Trp and the Arg399Gln polymorphisms (Moullan *et al*, 2003) and a protective effect for the T-C-G-G haplotype determined by all four *XRCC1* genetic polymorphisms, -77T>C, Arg194Trp, Arg280His, and Arg399Gln (Brem *et al*, 2006). Although the results appear divergent, the studies differ in the specific type(s) of adverse reactions being studied, the length of follow-up for side effects, and adjustment for patient-related factors; therefore, comparison of the findings is problematic. Polymorphisms in *XRCC3* and *APEX1* were studied in breast cancer patients receiving radiotherapy (summarised in Chistiakov *et al*, 2008; Popanda *et al*, 2008). Consistent with our null results, all of these studies failed to show a contribution of these SNPs to the risk of adverse reactions after radiotherapy, implying that they may not be promising candidates for predicting late radiosensitivity.

To our knowledge, this is the first epidemiological study on the two *TP53* genetic variants as predictors of late tissue reactions to radiation therapy. However, both the *TP53* codon 72 and intron 3 variants have been found to be associated with poorer prognosis of non-small cell lung cancer (Boldrini *et al*, 2008). Patients receiving chemoradiotherapy for advanced head and neck cancer were found to have higher response rates and survival when their tumors expressed the proapoptotic 72 Arg allele (Sullivan *et al*, 2004).

This study has a number of strengths. Breast cancer patients from this cohort were treated similarly, with radiation dosage carefully assessed, and patients were followed prospectively. Improved radiation techniques at the time of patient recruitment, as well as retrieval of individual irradiation dose methods and records, allowed for proper calculations of BED. The phenotype was precisely defined using the standardised scoring system for late toxicity. In addition, we accounted for patient- and treatment-related factors that influenced risk for telangiectasia when assessing the effect of the genetic variants.

Both telangiectasia and subcutaneous fibrosis are among the most common long-term skin side effects of radiation therapy.

Owing to differences in physiological response to radiation of the various skin layers involved and thereby possible differing genetic susceptibility, we opted to restrict the present analysis to telangiectasia because of the limited occurrence of fibrosis and therefore restricted power. Progressive nature of these complications, together with longer time to follow-up, may permit later analyses of late normal tissue complications in this cohort in the future.

In conclusion, this prospective study showed that variants in the *TP53* gene are associated with risk of late skin toxicity after accounting for patient-related factors and treatment modalities. As this is the first report on the involvement of p53 in late skin adverse effects, replication of these findings in other studies is encouraged. Advances in the search for biomarkers of radiation-induced late skin side effects may lead to improved treatment choices for breast cancer patients, and improve cosmetic outcome as well as quality of life after surviving breast cancer.

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