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No association between TGF-β1 polymorphisms and radiation-induced lung toxicity in a European cohort of lung cancer patients

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ABSTRACT

This study aimed at validating the previously published association between TGF-β1 single nucleotide polymorphisms and a reduced risk for radiation-induced lung toxicity. We were not able to confirm the reported association, neither using maximum dyspnea score nor after correction for baseline dyspnea score.

Radiation-induced lung toxicity (RILT) is a dose-limiting factor for radiotherapy [1]. Because current parameters are only moderately associated with RILT, there is a demand for novel parameters [2–4]. Yuan et al. [5] and Wang and Bi [6] have studied previously the association between TGF-β1 single nucleotide polymorphisms (SNPs; rs1800469:C-509T, rs1800470:T869C and rs1800471: G915C) and the risk of developing radiation-induced pneumonitis (RP) in patients with non-small-cell lung cancer (NSCLC). Yuan et al. showed an association between the CT/CC genotypes of rs1982073:T869C (new SNP ID rs1800470) and a lower RP risk (hazard ratio 0.489 for grade $\geqslant$2 and 0.390 for grade $\geqslant$3) in 164 patients of mixed ethnicity with TGF-β1 genotype distributions similar to Caucasian patients in general [5]. Wang and Bi could not confirm an association for the same three SNPs in a Chinese cohort (n = 178) and also did not find a correlation between any of the TGF-β1 SNPs and TGF-β1 levels in the blood [6]. The authors suggested that differences in genetic backgrounds between people of Caucasian and Chinese descent could partly explain the contradictory results.

Therefore, we genotyped the same three TGF-β1 SNPs in a European cohort of lung cancer patients.

Material and methods

Patient population

The patient population (n = 209) is described in Table 1. Patients were recruited from MAASTRO Clinic (n = 162) and the Ghent University Hospital (n = 47). All the treatments were controlled with in vivo dosimetry [7]. Lung toxicity was scored using the Common Terminology Criteria for Adverse Events version 3.0 for dyspnea before (baseline) and up to 1 year after (maximum) radiotherapy. The study was approved by the ethics committees of both centers, and all study participants provided written informed consent.

Genotyping

The TGF-β1 SNPs were genotyped using PCR and Sanger sequencing. The target fragments were amplified using primers 5′-GTC GCA GGG TGT TGA GTG ACA G-3′ and 5′-GGA CCA GGC GGA GAA GGC TTA-3′ for rs1800469 and 5′-ACC ACT GCG CCC TTC TCC CT-3′ and 5′-GCG CTT CCG CTT CAC CAG CT-3′ for rs1800470 + rs1800471 according to the following program: 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 58 °C for

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Table 1
Patient characteristics ($N = 209$).

Variable	No.		%
Sex			
Male	150		71.8
Female	59		28.2
Age, years			
Median		66	
Range		42–87	
Smoking status			
No smoker	7		3.3
Former smoker	128		61.2
Current smoker	69		33.0
Unknown	5		
Histopathology			
NSCLC, NOS	149		71.3
SCLC	52		24.9
NSCLC + SCLC	1		0.5
Unknown	7		
Tumor stage			
I	26		12.4
II	22		10.5
IIIa	72		34.4
IIIb	72		34.4
IV	5		2.4
Unknown	12		
Treatment			
Sequential chemoradiation	58		27.8
Concurrent chemoradiation	108		51.7
Radiation alone	42		19.6
Unknown	1		
Radiation dose (n = 209), Gy			
Median		60.0	
Range		40–79.2	
Mean lung dose (n = 186), Gy			
Median		14.4	
Range		2.6–23.4	
V_{20} (n = 108),%			
Median		23.4	
Range		0–54	
Pre-RT FEV 1.0 (n = 133), L			
Median		2.1	
Range		0.6–3.9	
Baseline dyspnea score			
0	110		52.6
1	68		32.5
2	27		12.9
3	4		1.9

Abbreviations: NSCLC, non-small-cell lung cancer; SCLC, small-cell-lung cancer; NOS, not otherwise specified; V_{20}, volume of normal lung receiving 20 Gy or more radiation; FEV 1.0, forced expiratory volume in 1 s.

45 s, 72 °C for 1 min, and a final extension step at 72 °C for 10 min. After evaluation in agarose gel, the PCR products were cleaned-up using a QIAquick PCR Purification Kit (QIAGEN). Then, the purified DNA is sequenced using a BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) and analyzed on a ABI3730 (Applied Biosystems) using the software program Sequencing Analysis 5.2 (Results in Supplementary Table). Genotypes were scored by two independent researchers, blinded for the dyspnea scores. Only sequences with high software quality scores (trace score > 20, <1% probability of error) and clear homozygous or heterozygous calls were included.

Statistical analysis

To test for significant correlations between genotypes (three separate or two combined versus the other) and maximum dyspnea score or delta dyspnea score, chi square tests were used with SPSS software. To check if dosimetric parameters were unevenly distributed between genotypes, we performed *T* tests and ANOVA.

Results

The values for radiation dose, mean lung dose and V_{20} of the patient population compare well with the Chinese study [6], whereas the mean lung dose and V_{20} were lower compared with the study by Yuan et al. [5]. A power calculation showed that we are able to detect differences in prevalence between genotypes of a magnitude of 15–20 percent points with this population of 209 patients (power 0.80; alpha 0.05; assuming equal division across subgroups) which is of the same magnitude as Yuan and colleagues [5]. Furthermore, our follow-up time of up to one year was similar to the 10 months follow-up by Yuan et al. for the reported significant hazard ratios. We failed to validate that any of the three TGF-β1 SNPs were associated with increased lung toxicity when using the patients' maximum dyspnea score (Table 2).

In addition to classifying the patients according to the maximum dyspnea score they developed after radiotherapy, we performed another analysis taking into account the dyspnea score of the patients before radiotherapy (delta dyspnea = maximum dyspnea − baseline dyspnea) to correct for lung disease before radiotherapy [1]. A similar approach, quantifying changes in regional perfusion between pre- and post-radiation, has recently been used by others [8]. Again we could not find an association between any of the three TGF-β1 SNPs and delta dyspnea score (Table 2). Statistical tests for differences in dosimetric parameters between genotypes were not significant.

Although we cannot exclude smaller differences than those which are detectable with the current population size, we certainly did not detect a large effect as described by Yuan et al. [5].

Discussion

We could not validate the previously published association between the CT/CC genotypes of SNP rs1800470 and a lower risk for radiation-induced lung toxicity (RILT) in a European cohort. Our results imply that the previous contradictory results concerning TGF-β1 SNPs and RILT risk cannot only be explained by ethnic differences in genetic background as they did not correspond with the results of the American/Caucasian population [5,6]. During the last decade, multiple studies have reported associations between TGF-β1 SNPs and radiation toxicity in multiple types of cancer [9–14]. However, a large ($N = 1613$) independent validation study within the Radiogenomics Consortium, the RAPPER study, and several other studies did not succeed in confirming these associations [15–17]. The major limitations of multiple studies seemed to be the small sample size, multiple testing issues and the absence of independent validation, resulting in false positive associations.

In conclusion, to find significant results for the TGF-β1 SNPs it appears that a sufficiently large number of patients is necessary, combined with external validation. Our results stress the importance of including corrections for baseline scores. Given the contrasting results obtained for the TGF-β1 SNPs so far, no definite role can be formulated for these polymorphisms in radiation-induced toxicity and they should not be used in treatment decision. New insights in the molecular pathology of RILT might help to identify novel specific parameters in the near future [18].

Conflict of interest statement

None.

Table 2
Correlation between *TGF-β1* genotypes and lung toxicity using maximum dyspnea score and delta dyspnea score.

Genotype	Maximum dyspnea score			Delta dyspnea score			
	Total no. of patients	No. max. dyspnea score ⩾ 2	*P*[a]	No. improve delta dyspnea score	No. worsen delta dyspnea score	*P* 3 group[a]	*P* improve + stable versus worsen[a]
rs1800469: C-509T							
CC	101	31		16	34		
CT	95	26	0.498	8	27	0.338	0.629
TT	13	2		2	5		
CT + TT	108	28	0.444	10	32	0.208	0.531
rs1800470: T869C							
TT	113	36		18	36		
TC	75	16	0.251	5	22	0.288	0.744
CC	21	7		3	8		
TC + CC	96	23	0.206	8	30	0.219	0.925
rs1800471: G915C							
GG	186	49		24	59		
GC	23	10	0.085	2	7	0.814	0.900
CC	0	0		0	0		
GC + CC	23	10	0.085	2	7	0.814	0.900

All three SNPs were also genotyped by Yuan et al. [5] and Wang and Bi [6], rs180470 corresponds to rs1982073 which received a new ID after the publication by Yuan et al.
[a] χ^2 test for three (grey) or two (combined) genotypes.

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