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ATM and *TGFB1* genes polymorphisms in prediction of late complications of chemoradiotherapy in patients with locally advanced cervical cancer

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The purpose of our study was to evaluate a possible correlation between genetic polymorphisms in *ATM* and *TGFB1* genes and late toxicity of chemoradiotherapy for locally advanced cervical cancer. Fifty five patients with FIGO stage IIB and higher without a disease recurrence with a mean follow up of 6 years were included. Late toxicity was assessed by EORTC/RTOG late toxicity criteria. Univariate and multivariate logistic regression model was used for statistical analysis. Degree of association between polymorphisms and late toxicity of chemotherapy was assessed on the basis of phi-coefficient (φ) as well. We did not find any association between 5557G>A polymorphism in the *ATM* gene or single *TGFB1* polymorphisms and late toxicity. *TGFB1* compound homozygosity (-1552delAGG, -509C>T, L10P) was a significant predictive factor of grade III-IV and any grade of complications in both univariate and multivariate logistic regression analyses and statistical significance of association between polymorphisms and late toxicity of chemoradiotherapy was confirmed also by the evaluation of phi-coefficient (φ). We conclude that haplotypes instead of single nucleotide polymorphic sites in the genes may better characterize the individual radiosensitivity.

Key words: cervical cancer, radiotherapy, ATM, TGFB1, late toxicity

Cervical cancer is the third most commonly diagnosed gynecological cancer in the Czech Republic. In 2008, the incidence was 19.2/100,000, with 311 deaths. The standard treatment method for locally advanced cervical cancers (International Federation of Gynecology and Obstetrics [FIGO] stage IIB and higher) includes combination of external beam radiotherapy (EBRT), brachytherapy (BRT) and concomitant chemotherapy. This treatment provides good tumor control, but also a risk of late complications in the irradiated area. Severe late complications affect 10–15 % of patients. They include mostly tissue fibrosis, necrosis, and mucous membrane atrophy, which primarily affects the rectum, bladder, and small intestine. In the worst cases, these complications may lead to perforation of the intestine and bleeding, or the formation of fistulae.

A part of patients treated by the same doses and technique of radiotherapy will develop complications. A possible explanation is increased individual radiosensitivity caused by genetic factors. From laboratory research it is known, that certain types of mice have a greater predisposition to develop typical late reactions after radiotherapy, such as postradiation fibrosis [1]. A congenital predisposition to a higher radiosensitivity was shown in patients with rare genetic syndromes such as ataxia telangiectasia, Fanconi anemia, and Bloom syndrome. Patients with these syndromes show not only increased clinical but also *in vitro* cell radiosensitivity. All these syndromes are associated with hereditary mutations of genes producing proteins important for the detection and repair of damaged DNA molecules [2]. Single nucleotide polymorphisms (SNPs) comprise approximately 90 % of all naturally occurring genome sequencing modifications [3], which can give rise to hundreds of variations in different genes. Finding associations between the occurrence of polymorphisms in the candidate genes and late post-radiation tissue toxicity could help to predict the development of late complications after radiotherapy.

The *ATM* gene and its protein product ATM kinase play an important role in cell cycle regulation. It is a principle regulator in the three checkpoints of the cell cycle and contributes to

DNA repair, cell cycle arrest, regulation of apoptosis and stress response. Its name was derived from the autosomal recessive disease known as ataxia telangiectasia. One of the signs of this disease is an increased tissue radiosensitivity. These patients are homozygous carriers of mutations in *ATM*, which was found on chromosome 11q23. Some studies proved a higher risk of late complications in patients with *ATM* 5557G>A polymorphism treated by radiotherapy [4, 5].

Transforming growth factor B1 (*TGFB1*) is a member of a multifunctional family of genes that encode growth factors acting as regulators of proliferation, apoptosis, differentiation, and extracellular matrix regeneration. *TGFB1* is located on chromosome 19q13 and its polymorphisms have been examined in connection with late radiotherapy toxicity. Kim de Ruyck et al. [6] found possible associations between *TGFB1* -1552delAGG, -509C>T, and L10P polymorphisms and late toxicity after radiotherapy in 78 patients treated for cervical or endometrial carcinoma, however the results were not statistically significant. The study also investigated the relationship between other polymorphisms in *TGFB1* (-800G>A, R25P, and T263I) and development of late complications, but with negative results.

In our retrospective study we attempted to elucidate relations between *ATM* 5557G>A polymorphism /rs1801516/ and four polymorphisms in the *TGFB1* gene (-1552delAGG /rs11466313/, -800G>A /rs1800468/,-509C>T /rs1800469/, L10P /rs1800470/), and late toxicity in patients treated for cervical carcinoma by chemoradiotherapy.

Patients and methods

Patients. The study included 55 patients with locally advanced cervical cancer (FIGO stage IIB and higher) treated by chemoradiotherapy at the Dept. of Oncology and Radiotherapy, University Hospital Hradec Králové, Czech Republic, between 2001–2010. None of the patients had disease recurrence. Basic demographic and clinical characteristics are described in Table 1. Patients were enrolled in the study successively as they come for follow up visit. The follow up was at least 6 months. The study was approved by ethical committee and all patients signed an informed consent.

Treatment and follow-up. All patients received concomitant chemoradiotherapy as a definitive treatment. EBRT was delivered to the pelvis by the four field BOX technique with parametrial boost, in cases with involvement of common iliac or paraaortic nodes the radiation fields were extended to the paraaortic region. During or after EBRT, patients received intrauterine BRT with a Fletcher three-channel applicator; a radiation dose was prescribed to the point A and doses to the rectal and urinary bladder points were calculated according to International Commission of Radiation Units and Measurements (ICRU) 38. In most patients weekly doses of cisplatin 40 mg/m^2 were used concomitantly with EBRT, in patients with impaired renal function cisplatin was replaced by weekly paclitaxel 50 mg/m^2. Because of the treatment protocol modification and individual condition of some patients (age, renal function) not all patients received the same dose of radiotherapy and type of chemotherapy. Treatment details are presented in Table1. The treatment parameters were included to the statistic evaluation.

After the end of the treatment patients were followed up in 3 month intervals during first 2 years, in 6 months intervals from 2 to 5 years and after that in 1 year intervals. Gynecological examination was performed during each visit, CT 1x/6 months and after 5 years 1x/year, chest X-ray 1x/year. In the case of complications the visits were more frequent.

Late complications after radiotherapy were evaluated according to the Radiation Therapy Oncology Group/European Organisation for Research and Treatment of Cancer (RTOG/EORTC) criteria. Patients were divided into three subgroups: subgroup without complications (subgroup 1, n=13), subgroup with grade I–II complications (subgroup 2, n=20), and subgroup with grade III–IV complications (subgroup 3, n=22). The grade I–II complications were proctitis and cystitis, the grade III–IV complications were bleeding, obstructions, formation of rectovaginal or vesicovaginal fistulae or intestine perforation.

DNA analysis. Two test tubes of anticoagulated blood were collected from each patient. From 200 μl of blood, DNA was extracted by micro-column method (QIAamp Mini Blood Kit, Qiagen, Germany). PCR reactions in the *ATM* and *TGFB1* genes were performed in the thermocycler ABI 2720 (Applied Biosystems, USA). Cycling conditions and sequences of primers for PCR amplification and restriction fragment length polymorphism (RFLP) in *ATM* 5557G>A polymorphic site we described previously [7]. Similar approach was chosen for analysis of -800G>A and -509C>T *TGFB1* polymorphisms

Table 1. Patients characteristics

Variables		Variables	No of cases
Age (mean)	47 years (31; 74)	**RT pelvis dose**	
Follow up (mean)	6 years (1; 10)	46/48.6 Gy	14
PS WHO	No of cases	50 Gy	41
0	35	**RT parametrium dose**	
1	17	14 Gy	38
2	1	9 Gy	17
3	2	**Paraaortic radiotherapy**	28
Histopathology		**BT dose**	
Spinocelular carcinoma	53	24 Gy	41
Adenocarcinoma	2	28/30 Gy	14
Grading		**Chemotherapy**	55
1	8	Cisplatin	45
2	32	Paclitaxel	10
3	15		
FIGO IIB	26		
FIGO IIIB	29		

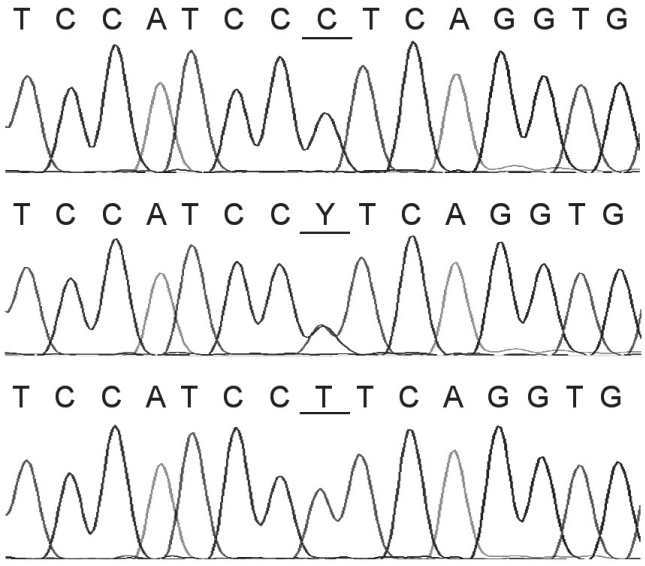

Figure 1. Sequencing analysis of *TGFB1* -509C>T genetic polymorphism (underlined). Upper part: wild-type genotype; middle part: heterozygous subject; lower part: -509T variant homozygous patient

[6]. The -1552delAGG polymorphism of the *TGFB1* promoter region was examined by fragmentation analysis (FA) in the ABI 3130 genetic analyzer (Applied Biosystems, USA), forward primer was modified at the 5′ end by 6-FAM fluorophore: 5'- FAM – CCA GGT GGA AGG TGG ATT AG – 3'; reverse primer: 5'- CTC CAG TCC CCA GGT AAC CAT C – 3'. Each RFLP or FA run contained control samples with the known genotype to ensure the validity of the obtained results. In first 20 examined specimens, the confirmatory sequencing with the same results was performed (Fig. 1). For L10P analysis, direct sequencing of amplicons [6] was carried out.

Statistical analysis. Logistic regression was used to analyse the relationship between patient characteristics or genetic markers and the grade of complications. The models were computed both as univariate for all predictors and multivariate for selected predictors that reached $p<0.05$ in univariate analysis. Results of statistical evaluations were accompanied by odds ratios (OR) with 95% confidence intervals. Degree of association between polymorphisms and late toxicity of chemoradiotherapy was assessed on the basis of phi-coefficient (φ) as well. Analyses were done with SPSS Statistics 19 (SPSS Statistics, 2010; IBM, USA).

The terms "compound heterozygote" and "compound homozygote" were used to indicate -1552delAGG/-509C>T/L10P linkage disequilibrium of these polymorphic sites.

Results

Genotype and allelic frequencies according to the patients groups are presented in Table 2. In the subgroup 3 there was a higher percentage of homozygous carriers of -1552 delAGG, -509C>T (18.2 % vs 7.7 %), and L10P (22.7 % vs 7.7 %) single allelic variants, and -1552delAGG/-509C>T/L10P) compound homozygotes in comparison with the subgroup 1.

In univariate logistic regression analysis of baseline characteristics and genetic markers vs degree of chronic toxicity the pelvic dose 50/60 Gy, parametrial irradiation to 14 Gy, radiotherapy of paraaortic lymphatic nodes, *TGFB1* -1552 delAGG, -509C>T and L10P single variant homozygotes and *TGFB1* compound homozygotes significantly correlated with grade III–IV late toxicity and any grade of complication. Statistical significance of association between polymorphisms and late toxicity of chemoradiotherapy was confirmed also by the evaluation of phi-coefficient (φ) (Table 3).

In multivariate analysis only compound homozygosity was a significant risk factor for grade III–IV and any grade of complications with p=0.021 and p=0.012, respectively (Table 3).

From clinical characteristics only a parametrial dose of 14 Gy was a risk factor for development of grade III–IV (p=0.006) and grade I–IV (p=0.004) complications (Table 3).

Discussion

In our study we tried to test correlation of possible clinical factors (doses, size of radiation fields, type of chemotherapy, age), and candidate genes polymorphisms (*ATM* 5557G>A polymorphism; -1552delAGG, -800G>A, -509C>T, and L10P polymorphisms in *TGFB1*) with a risk of late complications on a surviving group of patients treated with radical chemoradiotherapy for an inoperable stage of cervical carcinoma. Taking into account negative findings of De Ruyck study [6], we did not analyse either R25P (rs1800471) or T263I (rs1800472) polymorphic sites in the *TGFB1* gene. We also omitted -800G>A (rs1800468) polymorphism from statistical analysis due to small number of patients with -800A allelic variant (six -800A heterozygotes, none -800A homozygote subjects).

Among clinical factors, pelvic dose >50 Gy, parametrial boost of 14 Gy and extended field radiotherapy of paraaortic nodes were predicting factors for grade III–IV complications and any grade of complications in univariate logistic regression models but in multivariate analysis only boost of 14 Gy was statistically significant.

The 5557G>A polymorphism of *ATM* has been a subject of several studies that examined the development of late complications after radiotherapy with ambiguous results. Andreassen et al found a significant association between hetero- or homozygous carriership of 5557G>A and the development of subcutaneous fibrosis after radiotherapy [8]. A study of Damaraju et al [9], which included 83 patients treated with radiotherapy for localized prostate cancer, investigated the association between SNPs of several candidate genes. They found an association between 5557G>A and the development of late radiotherapy toxicity. Another study of Andreassen et al. did not prove an association between carriership of 5557G>A and the development of late complications in 120 breast

Table 2. Allele and genotype frequencies in patients groups

Alleles/genotypes	*ATM*	*TGFB1*			
	5557G>A	-1552	-800	-509	10
Group I					
allele wt	24 (92.3 %)	15 (57.7 %)	24 (92.3 %)	15 (57.7 %)	15 (57.7 %)
allele M	2 (7.7 %)	11 (42.3 %)	2 (7.7 %)	11 (42.3 %)	11 (42.3 %)
wt/wt	11 (84.6 %)	3 (23.1 %)	11 (84.6 %)	3 (23.1 %)	3 (23.1 %)
M/wt	2 (15.4 %)	9 (69. 2%)	2 (15.4 %)	9 (69.2 %)	9 (69.2 %)
M/M	0 (0.0 %)	1 (7.7 %)	0	1 (7.7 %)	1 (7.7 %)
-1552/-509/10 het			9 (69,2%)		
-1552/-509/10 hom			1(7.7%)		
Other genotypes			3 (23.1%)		
Group II					
allele wt	36 (90.0 %)	32 (80.0 %)	37 (92.5 %)	32 (80.0 %)	27 (67.5 %)
allele M	4 (10.0 %)	8 (20.0 %)	3 (7.5 %)	8 (20.0 %)	13 (32.5 %)
wt/wt	17 (85.0 %)	13 (65.0 %)	17 (85.0 %)	13 (65.0 %)	9 (45.0 %)
M/wt	2 (10.0 %)	6 (30.0 %)	3 (15.0 %)	6 (30.0 %)	9 (45.0 %)
M/M	1 (5.0 %)	1 (5.0 %)	0	1 (5.0 %)	2 (10.0 %)
-1552/-509/10 het			5(25%)		
-1552/-509/10 hom			1(5%)		
Other genotypes			14(70%)		
Group III					
allele wt	40 (90.9 %)	30 (68.2 %)	43 (97.7 %)	30 (68.2 %)	27 (61.4 %)
allele M	4 (9.1 %)	14 (31.8 %)	1 (2.3 %)	14 (31.8 %)	17 (38.6 %)
wt/wt	18 (81.8 %)	12 (54.5 %)	21 (95.5 %)	12 (54.5 %)	10 (45.5 %)
M/wt	4 (18.2 %)	6 (27.3 %)	1 (4.5 %)	6 (27.3 %)	7 (31.8 %)
M/M	0 (0.0%)	4 (18.2%)	0 (0.0%)	4(18.2%)	5 (22.7%)
-1552/-509/10 het			5(22.7%)		
-1552/-509/10 hom			4(18.2%)		
Other genotypes			13(59.1%)		
Group II + III					
allele wt	76 (90.5 %)	62 (73.8 %)	80 (95.2 %)	62 (73.8 %)	54 (64.3 %)
allele M	8 (9.5 %)	22 (26.2 %)	4 (4.8 %)	22 (26.2 %)	30 (35.7 %)
wt/wt	35 (83.3 %)	25 (59.5 %)	38 (90.5 %)	25 (59.5 %)	19 (45.2 %)
M/wt	6 (14.3 %)	12 (28.6 %)	4 (9.5 %)	12 (28.6 %)	16 (38.1 %)
M/M	1 (2.4 %)	5 (11.9 %)	0	5 (11.9 %)	7 (16.7 %)
-1552/-509/10 het			10 (23.8%)		
-1552/-509/10 hom			5(11.9%)		
Other genotypes			27(64.3%)		

M = mutation
Wt = wild type
1552/-509/10 het = compound heterozygotes for 1552delAGG, -509C>T, and L10P
1552/-509/10 hom = compound homozygotes for 1552delAGG, -509C>T, and L10P

cancer patients with adjuvant radiotherapy after mastectomy [10]. Also we did not find 5557G>A polymorphism to be a predictive factor for chronic toxicity after radiotherapy in patients treated for advanced cervical cancer.

Fibrosis plays an important role in the development of late reactions after radiotherapy. The TGFB1cytokine has been demonstrated to be a key mediator of fibrogenesis in number of pathologic conditions, including postradiation tissue reactions [11]. Numerous studies reporting the association between high TGFB1 plasma levels in cancer patients and the development of severe radiation-induced fibrosis demonstrated the involvement of TGFB1 and its functional polymorphisms in clinical radiosensitivity. Theses studies included patients treated by radiotherapy for lung cancer [12, 13], prostate 14], head and neck cancer [15], breast cancer [16, 17], rectal cancer [18]. However, other studies have been somewhat confusing [19].

TGFB1 peptide is a product of *TGFB1* gene and some genotype changes can alter TGFB1 production. De Ruyck et al [6] investigated the association between six *TGFB1* polymorphisms (-1552delAGG, -800G>A, -509C>T, L10P, R25P, T263I) and

Table 3. Prediction of complication grade in univariate and multivariate logistic regression models

	Grade III–IV complications vs. no complications[1]			Any grade complications vs. no complications[1]		
	OR (95% CI)	*p* uni	*p* multi	OR (95% CI)	*p* uni	*p* multi
Age at treatment (years)	1.018 (0.944;1.099)	0.643		0.993 (0.929;1.060)	0.826	
Age category 41–50[2]	1.350 (0.211;8.617)	0.751		0.700 (0.138;3.558)	0.667	
Age category >50[2]	1.350 (0.211;8.617)	0.751		0.800 (0.159;4.023)	0.787	
BMI	1.014 (0.876;1.172)	0.856		0.988 (0.874;1.117)	0.848	
BMI category ≤24.9[3]	1.806 (0.391;8.348)	0.449		1.970 (0.495;7.832)	0.336	
BMI category >29.9[3]	1.250 (0.146;10.699)	0.839		1.136 (0.162;7.995)	0.898	
EBRT pelvis 50/60 Gy[4]	33.600 (3.381;333.885)	**0.003**		9.600 (2.338;39.423)	**0.002**	
EBRT parametrium 14 Gy[5]	14.250 (2.619;77.542)	**0.002**	**0.006**	9.562 (2.341;39.060)	**0.002**	**0.004**
EBRT PALU	4.815 (1.027;22.571)	**0.046**		4.902 (1.173;20.479)	**0.029**	
CHT CDDP	0.833 (0.068;10.202)	0.887		1.667 (0.139;20.014)	0.687	
CHT paclitaxel	1.618 (0.266;9.852)	0.602		1.294 (0.238;7.028)	0.765	
***TGFB1* -1552/-509**[7] **homozygous**[8]	5.988 (1.332;27.027) (φ=0.410; p=0.015)	**0.020**		5.618 (1.451;21.739) (φ = 0.356; p=0.008)	**0.012**	
***TGFB1* L10P homozygous**[8]	4.831 (1.096;21.277) (φ=0.363; p=0.032)	**0.037**		3.650 (0.964;13.889) (φ = 0.266; p=0.049)	0.056	
***TGFB1* compound** [9] **homozygous**[8]	7.634 (1.634;35.714) (φ=0.459; p=0.007)	**0.010**	**0.021**	7.194 (1.821;28.571) (φ = 0.406; p=0.003)	**0.005**	**0.012**

[1]Odds ratio (OR, supplied with 95% confidence interval) for univariate (p uni) models based on logistic regression; multivariate (p multi) regression based on backward stepwise selection algorithm.
[2]Reference category is ≤40 years.
[3]Reference category is BMI 25–29.9.
[4]Reference category is 46/48.6 Gy; EBRT of pelvis 50/60 Gy is identical to BRT 18/24 Gy with reference category BRT 28/30 Gy (patients who received EBRT of pelvis 50/60 Gy also received BRT 18/24 Gy.
[5]Reference category is 9 Gy EBRT of parametrium.
[7]*TGFB1* -1552delAGG and -509C>T polymorphisms are in perfect linkage equilibrium [6].
[8]Reference categories are wild-type homozygotes and heterozygotes.
[9]-1552delAGG/-509C>T/L10P *TGFB1* compound homozygotes
BMI = body mass index
CHT = chemotherapy
PALU – paraaortic EBRT

the occurrence of late normal tissue reactions after gynecologic radiotherapy in 78 women with cervical or endometrial cancer. The reason for screening of these polymorphisms is their possible contribution to the genetic control of TGFB1 plasma levels. The -1522delAGG, -800G>A, and -509C>T polymorphisms are located in the 5‘ end of the *TGFB1* promoter region and could affect the production of the cytokine. Both L10P and R25P polymorphisms are located in the signal sequence, which is responsible for the export of the newly synthesized protein across the membranes of the endoplasmatic reticulum. The T263I is possibly involved in the stability and activity of the protein. The study showed that *TGFB1* -1552delAGG, -509C>T, and L10P homozygosity may be associated with severe clinical radiosensitivity after gynecological radiotherapy. -1522delAGG/-509C>T/L10P homozygous patients had a 2.4 (p=0.347) times increased risk for developing moderate or severe RT reactions and a 3.6 (p=0.26) times increased risk to develop severe RT reactions. However, the results were not statistically significant using late reactions in the pelvis as endpoint.

In our study, *TGFB1* -1552delAGG, -509C>T, and L10P homozygous variants were significantly associated with a risk of grade III–IV and of any grade of complications in univariate logistic regression analysis, but not in multivariate analysis. On the other hand, *TGFB1* -1522delAGG/-509C>T/L10P compound homozygotes were significantly associated with grade III-IV and any grade of complications in both univariate and multivariate logistic regression models. In our study there is higher percentage of patients with severe late reaction (40 %) in comparison with the De Ruyck study (14 %). The explanation could be, that we examined patients in order they occurred for a visit and women with complications had more frequent appointments.

In our study we tried to evaluate a role of *ATM* and *TGFB1* in late toxicity of chemoradiotherapy for cervical cancer because the combined treatment is a standard approach in this diagnosis. Platinum based chemotherapy itself induces bulky DNA damage with ATM and TGFB1 responses [23]. One of explanations of chemotherapy related radiosensitation is inhibition of repair of radiation damage due impairment of DNA damage response pathway including mutations in responsible genes. Several studies supporting mutagenic effects of cisplatin [24, 25]. were previously published.

Our observation that single nucleotide polymorphism of *TGFB1* does not correlate with normal tissue radiosensitivity corresponds with results of recent studies [20, 21]. We think that the finding the statistically significant relationship between compound homozygosity and late complications, even in the small patients group, is interesting and deserves further evaluation. It seems, that haplotypes instead of single SNPs could be more helpful to characterize the individual radiosensitivity if *TGFB1* is assumed to be a key player [22]. It could be a small step towards better understanding of a complex multi-factorial process of radiation fibrosis.

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References

[1] JOHNSTON CJ, WILLIAMS JP, OKUNIEFF P, FINKELSTEIN JN. Radiation-induced pulmonary fibrosis: examination of chemokine and chemokine receptor families. Radiat Res 2002; 157: 256–265. http://dx.doi.org/10.1667/0033-7587(2002)157[0256:RIPFEO]2.0.CO;2

[2] GATTI RA. The inherited basis of human radiosensitivity. Acta Oncol. 2001; 40: 702–711. http://dx.doi.org/10.1080/02841860152619115

[3] ANDREASSEN CN, ALSNER J, OVERGAARD J. Does variability in normal tissue reactions after radiotherapy have a genetic basis--where and how to look for it? Radiother Oncol. 2002; 64: 131–140. http://dx.doi.org/10.1016/S0167-8140(02)00154-8

[4] IANNUZZI CM, ATENCIO DP, GREEN S, STOCK RG, ROSENSTEIN BS. ATM mutations in female breast cancer patients predict for an increase in radiation-induced late effects. Int J Radiat Oncol Biol Phys. 2002; 52: 606–613. http://dx.doi.org/10.1016/S0360-3016(01)02684-0

[5] CESARETTI JA, STOCK RG, LEHRER S, ATENCIO DA, BERNSTEIN JL. ATM sequence variants are predictive of adverse radiotherapy response among patients treated for prostate cancer. Int J Radiat Oncol Biol Phys. 2005; 61: 196–202. http://dx.doi.org/10.1016/j.ijrobp.2004.09.031

[6] DE RUYCK K, VAN EIJKEREN M, CLAES K, BACHER K, VRAL A. TGF beta1 polymorphisms and late clinical radiosensitivity in patients treated for gynecologic tumors. Int J Radiat Oncol Biol Phys. 2006; 65: 1240–1248. http://dx.doi.org/10.1016/j.ijrobp.2006.03.047

[7] BERANEK M, DRASTIKOVA M, PETERA J.: Polyadenylated sequence primers enable complete readability of PCR amplicons analyzed by dideoxynucleotide sequencing. Acta Medica 2012; 55: 160 – 164.

[8] ANDREASSEN CN, OVERGAARD J, ALSNER J, OVERGAARD M, HERSKIND C. ATM sequence variants and risk of radiation-induced subcutaneous fibrosis after postmastectomy radiotherapy. Int J Radiat Oncol Biol Phys. 2006; 64: 776–783. http://dx.doi.org/10.1016/j.ijrobp.2005.09.014

[9] DAMARAJU S, MURRAY D, DUFOUR J, CARANDANG D, MYREHAUG S. Association of DNA repair and steroid metabolism gene polymorphisms with clinical late toxicity in patients treated with conformal radiotherapy for prostate cancer. Clin Cancer Res. 2006 ; 12: 2545–2554. http://dx.doi.org/10.1158/1078-0432.CCR-05-2703

[10] ANDREASSEN CN, ALSNER J, OVERGAARD M, SORENSEN FB, OVERGAARD J. Risk of radiation-induced subcutaneous fibrosis in relation to single nucleotide polymorphisms in TGFB1, SOD2, XRCC1, XRCC3, APEX and ATM – a study based on DNA from formalin fixed paraffin embedded tissue samples. Int J Radiat Biol. 2006; 82: 577–586. http://dx.doi.org/10.1080/09553000600876637

[11] FINKELSTEIN JN, JOHNSTON CJ, BAGGS R, RUBIN P. Early alterations in extracellular matrix and transforming growth factor beta gene expression in mouse lung indicative of late radiation fibrosis. Int J Radiat Oncol Biol Phys. 1994; 28: 621–631. http://dx.doi.org/10.1016/0360-3016(94)90187-2

[12] GUERRA JL, GOMEZ D, WEI Q, LIU Z, WANG LI-E, et al. Association between single nucleotide polymorphisms of the transfotming growth factor β1 gene and the risk of severe radiation esophagitis in patients with lung cancer. Radiat Oncol 2012; 205, 299–304.

[13] STENMAK MH, CAI XW, SHEDDEN K, HAYMAN JA, YUAN SH. Combining physical and biologic parameters to predict radiation-induced lung toxicity in patients with non-small-cell lung cancer treated with definitive radiation therapy. Int J Radiat Oncol Biol Phys 2012; 84, 217–222. http://dx.doi.org/10.1016/j.ijrobp.2012.03.067

[14] PETERS CA, STOCK RG, CESARETTI JA, ATENCIO DP, PETERS S, et al. TGFB1 single nucleotide polymorphisms are associated with adverse quality of life in prostate cancer patients treated with radiotherapy. Int J Radiat Oncol Biol Phys 2008; 70, 752–759. http://dx.doi.org/10.1016/j.ijrobp.2007.05.023

[15] FELTL D, ZAVADOVA E, PALA M, HOZAK P. Posttreatment plasma transforming growth factor beta 1 [TGF-beta 1) level predicts for late morbidity in patients with advanced head and neck cancer. Neoplasma 2005; 52, 393–397.

[16] QUARMBY S, FAKHOURY H, LEVINE E, BARBER J, WYLIE J. Association of transforming growth factor beta-1 single nucleotide polymorphisms with radiation-induced damage to normal tissues in breast cancer patients. Int J Radiat Biol. Phys 2003; 79: 137–143.

[17] GIOTOPOULOS G, SYMONDS RP, FOWERAKER K, GRIFFIN M, PEAT I. The late radiotherapy normal tissue injury phenotypes of telangiectasia, fibrosis and atrophy in breast cancer patients have distinct genotype-dependent causes. Br J Cancer 2007; 96: 1001–1007. http://dx.doi.org/10.1038/sj.bjc.6603637

[18] SCHIRMER MA, MERGLER CPN, RAVE −FRANK M, HERRMANN MK, HENNIES S. Acute toxicity of radiochemotherapy in rectal cancer patients: a risk perticularly for carriers of the TGFB1 Pro25 variant. Int J Radiat Oncol Biol Phys 2012, 83, 149–157. http://dx.doi.org/10.1016/j.ijrobp.2

[19] BARNETT GC, COLES CE, BURNET NG, PHAROAH PD, WILKINSON J. No association between SNPs regulating TGF- β1 secretion and late radiotherapy toxicity to the breast: results from the RAPPER study. Radiother Oncol, 2010, 97, 9–14. http://dx.doi.org/10.1016/j.radonc.2009.12.006

[20] BARNETT GC, ELLIOTT R, ALSNER J, ANDREASSEN CN, ABDELHAY O. Individual patients data meta-amalysis shows no association betweem the SNP rs180469 in TGFB and late radiotherapy toxicity. Radiother Oncol 2012, 105, 289–295. http://dx.doi.org/10.1016/j.radonc.2012.10.017

[21] VOETS AM, OBERIJE C, STRUIJK R, REYMEN B, DE RUYCK K. No associatoon between TGFB1 polymorphisms and radiation-induced lung toxicity in a European cohort of lung cancer patients. Radiother Oncol 2012, 105, 296–298. http://dx.doi.org/10.1016/j.radonc.2012.09.016

[22] SCHIRMER M, BROCKMOLLER J, RAVE-FRANK M, VIRSIK P, WILKEN B. A putatively functional haplotype in the gene encoding transforming growth factor beta-1 as a potential biomarker for radiosensitivity. Int J Radiat Oncol Biol Phys 2011, 79, 866–874. http://dx.doi.org/10.1016/j.ijrobp.2010.08.040

[23] WOODS D, TURCHL JJ_ Chemotherapy induced DNA damage responses. Cancer Biology and Therapy, 2013, 14, 1–11. http://dx.doi.org/10.4161/cbt.23761

[24] SOLARY E, PLENCHETTE S, SORDET O, REBE C, DUCOROY P, et al.: Modulation of apoptotic pathways triggered by cytotoxic agents. Therapie 2001, 56, 511–518.

[25] CARIELLO NF, SWENBERG JA, SKOPEK TR: In vitro mutational specifity of cisplatin in the human hypoxanthine guanine phosphoribosyltransferase gene 1. Cancer Research 1992, 52, 2866–2873.