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Significance of an exon 2 G4C14-to-A4T14 polymorphism in the *p73* gene on survival in  
rectal cancer patients with or without preoperative radiotherapy

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## Abstract

**Background and Purpose:** An exon 2 G4C14→A4T14 polymorphism in the p73 gene was shown to be related to survival in several types of cancers, including colorectal cancer. The purpose was to investigate if this polymorphism was related to survival in rectal cancer patients with or without preoperative radiotherapy.

**Material and Methods:** DNA extracted from tissue of 138 rectal cancer patients that received preoperative radiotherapy or had surgery alone was typed for the polymorphism by PCR using confronting two-pair primers.

**Results:** Among patients, 69% had GC/GC genotype, 27% GC/AT and 4% AT/AT. In the radiotherapy group, patients carrying the AT (GC/AT+AT/AT) allele had stronger expression of p53 ( $p=0.001$ ) and survivin protein ( $p=0.03$ ) than those carrying the GC/GC genotype. Further, among patients receiving preoperative radiotherapy the GC/GC genotype tended to be related to better survival ( $p=0.20$ ). Patients with GC/GC genotype, along with negative p53 and weak survivin expression showed better survival than the other patients ( $p=0.03$ ), even after adjusting for TNM stage and tumor differentiation ( $p=0.01$ , RR, 7.63, 95% CI, 1.50-38.74). In the non-radiotherapy group, the polymorphism was not related to survival ( $p=0.74$ ).

**Conclusions:** Results suggest that the p73 G4C14→A4T14 polymorphism could be one factor influencing outcome of preoperative radiotherapy in rectal cancer patients.

## Introduction

Colorectal cancer is a common malignancy with about one million new cases diagnosed world-wide every year. In 2002 colorectal cancer represented 9.4 % of the world total cancer, and was ranked the fourth most common cancer in men and third in women. Approximately one third of colorectal carcinomas are situated in the rectum [1]. The mortality rate in rectal cancer is about 50% [1]. Preoperative radiotherapy has reduced recurrence rates and improved survival [2], but this treatment has some adverse effects. Therefore, predictive factors are needed to identify patients benefiting from radiotherapy.

The p73 protein is encoded by a gene, *TP73*, located to chromosome 1p36, a region frequently deleted in various cancers [3]. The structure and function of the p73 protein is homologous to p53, and because of these similarities, p73 can activate the transcription of p53-responsive genes and inhibit cell growth in a p53-like manner by inducing apoptosis [4]. However, p73 does not seem to be a classical tumor suppressor. Unlike mice lacking p53, p73-deficient mice do not develop spontaneous tumors, although they display other abnormalities, such as defective neurogenesis and abnormal reproductive and social behaviour [5]. p73 is rarely mutated in primary tumors [6], rather, p73 is overexpressed in various tumor types [7, 8], compared with normal tissues. This suggests that p73 plays an oncogenic role in tumorigenesis. A high expression of the p73 protein has been found to be associated with a poor prognosis in several types of cancers, including colorectal cancer [8].

Some single-nucleotide polymorphisms have been found in the p73 gene, but none of them cause an amino acid substitution. One of the allelic polymorphisms of p73 consists of a double nucleotide substitution at positions 4 and 14 of exon 2 (G4C14 → A4T14), just upstream of the initial start codon. The polymorphism is not located in the coding sequence of the exon, but occurs in a region of the transcript that could theoretically

form a stem-loop structure, possibly affecting gene expression. Since the two nucleotide substitutions are in complete linkage disequilibrium, there are three genotypes: GC/GC, AT/AT and GC/AT [3].

The role of the p73 G4C14→A4T14 polymorphism in the risk of developing cancer has been investigated in several studies [9-17], but the results are inconsistent, varying between types of cancers and populations. It has previously been found that the AT/AT genotype may be associated with a higher risk of developing colorectal cancer, but also that patients carrying the AT allele has a better prognosis [18].

In the present study, we investigated whether the G4C14→A4T14 polymorphism of the p73 gene was related to survival in rectal cancer patients who received or did not receive preoperative radiotherapy, and whether there were any relationships of the polymorphism with clinicopathological variables and expression of other proteins.

## Materials and Methods

### Patients

This study included the patients with rectal adenocarcinoma from the Southeast Swedish Health Care region who participated in a Swedish clinical trial of preoperative radiotherapy between 1987 and 1990 [2]. Surgical specimens were obtained by either rectal amputation or anterior resection from 138 patients (80 men and 58 women). The mean age at diagnosis was 66 years (range 38-85). The mean follow-up time was 86 months (range 0-193). Sixty-five patients were randomized to preoperative radiotherapy, receiving 25 Gy in 5 fractions over a median of 6 days (range 5-12). Surgery was performed after a median of 3 days (range 1-13) after radiotherapy. Seventy-three patients had surgery alone. The characteristics of the patients and tumors are given in Table 1. Number of malignancies included those malignancies which developed before, synchronously and/or after the present rectal cancer.

### Immunohistochemistry of p73, p53 and survivin

The data regarding the expression of p73 [19], p53 [20] and survivin [21], on the same surgical material from both the non-radiotherapy and radiotherapy groups as used in the present study (not biopsies), were taken from previous studies performed at our laboratory. Immunohistochemical staining for the three proteins was performed on 5 µm sections from paraffin-embedded surgical specimens. The sections were incubated in an oven, then deparaffinized in xylene and hydrated in descending concentrations of ethanol. The sections were put in citrate buffer (pH 6.0) and boiled in a microwave oven to unmask epitopes, followed by washing in phosphate buffered saline (PBS). Endogenous peroxidase activity was inhibited with 3% H<sub>2</sub>O<sub>2</sub> in methanol. After blocking (goat ABC staining system, sc-2023, Santa Cruz Biotechnology, Santa Cruz, CA for p73; normal rabbit serum for p53; and serum-

<i>Table 1. Characteristics of patients and tumors</i>		
<b>Characteristics</b>	<b>Non-Radiotherapy <i>n</i> (%)</b>	<b>Radiotherapy <i>n</i> (%)</b>
<i>Gender</i>		
Male	40 (55)	40 (62)
Female	33 (45)	25 (38)
<b>Age (years)</b>		
≤66	24 (33)	29 (45)
>66	49 (67)	36 (55)
<i>TNM stage</i>		
I	19 (26)	18 (28)
IIA	19 (26)	22 (34)
IIIA	8 (11)	1 (2)
IIIB	10 (14)	14 (22)
IIIC	13 (18)	4 (6)
IV	4 (5)	6 (9)
<i>Differentiation</i>		
Good	4 (5)	5 (8)
Moderate	53 (73)	41 (63)
Poor	16 (22)	19 (29)
<i>Number of malignancies*</i>		
Single	63 (86)	54 (83)
Multiple	8 (11)	11 (17)
Unknown	2 (3)	0
<i>Surgical type</i>		
Rectal amputation	39 (53)	27 (42)
Anterior resection	34 (47)	38 (58)
<i>Resection margin</i>		
Tumor free	69 (95)	60 (92)
Tumor	4 (5)	5 (8)
<b>To anal verge, Mean (cm)</b>	<b>7</b>	<b>8</b>

\*There were other colorectal cancers and/or other types of malignancies before, synchronously or after the present rectal cancer.

free protein block, Dako, Carpinteria, CA for survivin), a primary polyclonal goat antibody against p73 (Santa Cruz Biotechnology), or a monoclonal antibody PAb 1801 against p53 (Oncogene Science, Manhasset, New York), recognizing an epitope found in both wild type

and mutant p53 protein, or a monoclonal antibody against survivin (ab-1 clone 8 E2, Neomarkers, Westinghouse, CA) was added to the sections for incubation at 4°C over night (for p73 and survivin) or 30 min at room temperature (for p53). For the detection of p73, a biotinylated secondary antibody (Santa Cruz Biotechnology) was used. For color development an AB enzyme reagent, containing avidin and biotinylated horseradish peroxidase, was added followed by peroxidase substrate (Santa Cruz Biotechnology) containing 3,3'-diaminobenzidine chromogene, peroxidase substrate and buffer. For p53, a secondary antibody (mouse immunoglobulin, Dakopatts, Glostrup, Denmark) was applied for 30 min, followed by washing and incubation for 30 min with mouse peroxidase-antiperoxidase. For survivin, sections were incubated with a biotinylated rabbit anti-mouse immunoglobulin (Dakopatts) and peroxidase-conjugated streptavidine (Dakopatts) for 30 min each, separated by washing in PBS. For both p53 and survivin the peroxidase reaction was performed with 3,3'-diaminobenzidine (Sigma Chemical, St. Louis, MO) and H<sub>2</sub>O<sub>2</sub>. All sections were then counterstained with haematoxylin. A negative control was added in every run, where PBS was used instead of the primary antibody.

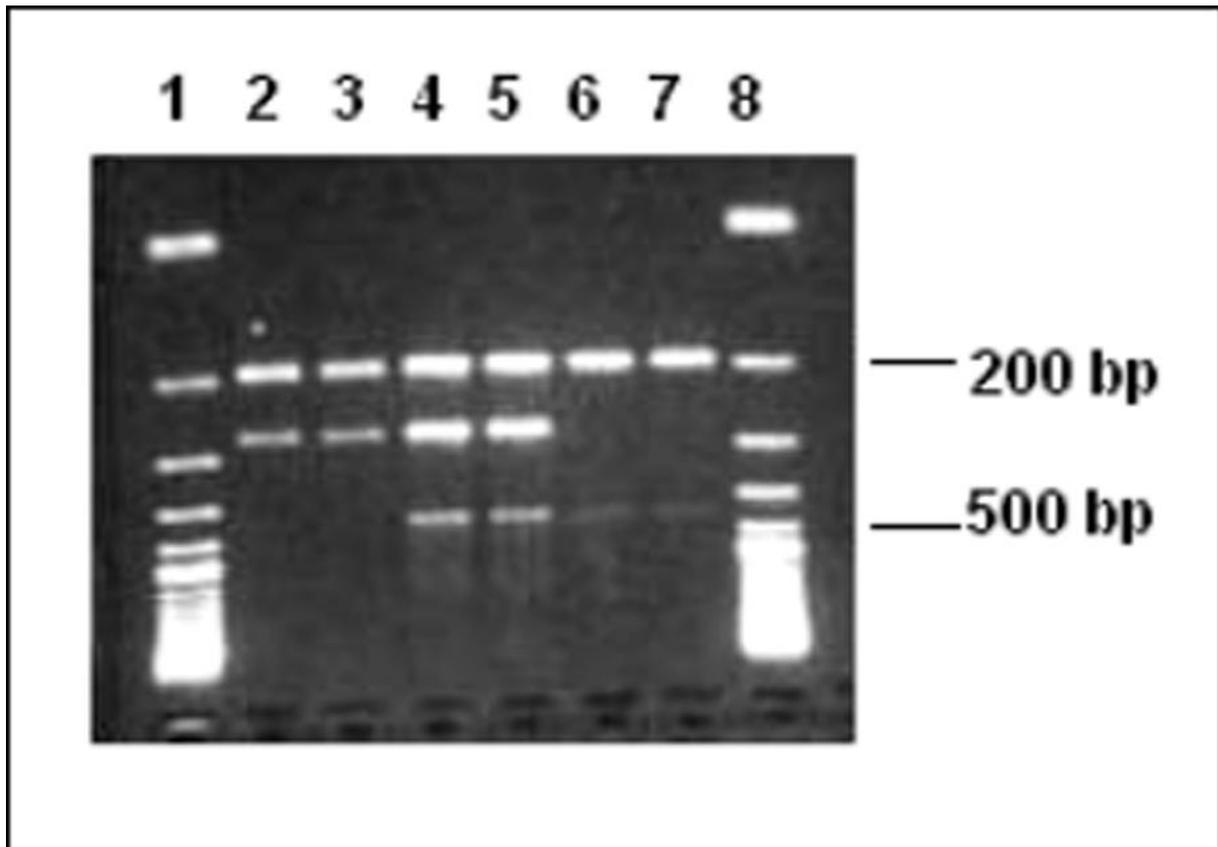
Staining patterns for p73 included cytoplasmic and nuclear staining, for p53 only nuclear staining and for survivin only cytoplasmic staining. For nuclear expression the staining was graded as negative (<5% of positive cells) and positive. For cytoplasmic expression the staining was graded as negative (<5% of positive cells), weak, moderate and strong. In the statistical analysis negative staining and weak staining were combined in one group called weak, and moderate staining and strong staining in another group called strong. In all cases the stained sections were evaluated in a blinded fashion by two independent investigators, without any knowledge of the clinicopathological data. In cases with discrepant scoring the cases were re-examined until consensus was reached. Cells in areas with necrosis, poor morphology or in section margins were not counted.

## DNA-extraction

Genomic DNA was extracted from 50 µm paraffin embedded tissue sections from rectal cancer patients. We used surgically removed normal lymph nodes ( $n = 25$ ), normal mucosa ( $n = 28$ ) from the distant margin of distant resection, and tumor samples ( $n = 85$ ). The Gentra Puregene Tissue Kit (Qiagen, Minneapolis, MN) was used according to the manufacturer's instructions. The concentration and purity of the DNA were measured spectrophotometrically.

## PCR

The patients were typed for the *p73* G4C14→A4T14 genotype by PCR using confronting two-pair primers. The PCR was performed in a reaction volume of 20 µl, containing 50 ng DNA, 1 x PCR buffer, 0.2 mM dNTP-mix, 0.25 U/µl ThermoWhite Taq DNA Polymerase, (Saveen & Werner, Malmö, Sweden) and 0.5 µM of each of the four primers (Sigma-Genosys, Cambridge, UK). The AT allele was amplified using primers F1 [5'CCACGGATGGGTCTGATCC3'] and R1 [5'GGCCTCCAAGGGCAGCTT3'] producing a 270 bp fragment. The GC allele was amplified using primers F2 [5'CCTTCCTTCCTGCAGAGCG3'] and R2 [5'TTAGCCCAGCGAAGGTGG3'], producing a 193 bp fragment. Primers F1 and R2 also produces a 428 bp fragment common to all PCR-runs. The PCR was performed under the following conditions: 10 minutes of initial denaturation at 95 °C, followed by 35 cycles of 95 °C for 1 minute, 60 °C for 45 seconds, 72 °C for 1 minute and a final extension at 72 °C for 5 minutes. The PCR products were resolved on a 2% agarose gel containing ethidium bromide (~0.5 µg/ml) and visualized in UV-light (Fig. 1.).



*Fig. 1. Detection of the p73 G4C14→A4T14 with PCR and confronting two-pair primers. The gel shows: lane 1 and 8 for 100 bp ladder; lanes 2-5 for GC/AT and lanes 6-7 for GC/GC. The F1 and R2 primer product is only clearly visible in lanes 4-5.*

### Statistical analysis

The  $\chi^2$  test was used to determine relationship of the p73 genotype, regarding the G4C14→A4T14 polymorphism, with different variables. Cox's proportional hazard model was used to test relationship between p73 genotype and patients' survival. The Kaplan-Meier method was used to calculate survival curves. Because of the low number of patients with the AT/AT genotype, the AT/AT genotype was grouped together with the GC/AT genotype in the statistical analysis. All statistical analysis was done in Statistica 7.0. Two-sided p-values of less than 0.05 were considered statistically significant.

## Results

### Genotype frequencies of the p73 polymorphism

The p73 genotype regarding the G4C14→A4T14 polymorphism was examined in 138 rectal cancer patients. Among the patients, 95 (69%) were wild type (GC/GC), 37 (27%) were heterozygotic (GC/AT) and 6 (4%) were homozygotic (AT/AT). Neither of the genotypes was related to any of the clinicopathological variables, such as gender, age, complication, local or distant recurrence, differentiation, inflammatory infiltration, necrosis or fibrosis (data not shown).

### The p73 polymorphism in relation to p53 and survivin expression

In the radiotherapy group, the patients carrying the AT allele (hetero – and homozygotic combined) more frequently had positive p53 expression in their tumors than the patients with the GC/GC genotype ( $p=0.001$ , Table 2). In the non-radiotherapy group there was no difference in p53 expression between the patients carrying the AT allele and the patients with the GC/GC genotype ( $p=0.73$ , Table 2).

The same was the case with the expression of survivin in the tumors. In the radiotherapy group, the patients with the AT allele had a significantly higher frequency of strongly expressed survivin in tumors than those with GC/GC genotype ( $p=0.03$ , Table 2). Whereas, there was no relationship seen in the non-radiotherapy group ( $p=0.78$ , Table 2)

### The p73 polymorphism in relation to disease-free survival

In the overall group of patients, there was no relationship between the p73 G4C14→A4T14 polymorphism and disease-free survival ( $p=0.54$ , data not shown). In the non-radiotherapy

**Table 2.** p73 polymorphism in relation to p53 and survivin expression in rectal cancer patients with and without preoperative radiotherapy

	Non-radiotherapy			Radiotherapy		
	GC/GC <i>n</i> (%)	GC/AT+AT/AT <i>n</i> (%)	<i>p</i>	GC/GC <i>n</i> (%)	GC/AT+AT/AT <i>n</i> (%)	<i>p</i>
<b>p53</b>						
Negative	44 (88)	17 (85)	0.73	41 (98)	14 (70)	0.0013
Positive	6 (12)	3 (15)		1 (2)	6 (30)	
<b>Survivin</b>						
Weak	16 (48)	8 (44)	0.78	15 (60)	2 (20)	0.032
Strong	17 (52)	10 (56)		10 (40)	8 (80)	

group there was no relationship of the polymorphism with survival either ( $p=0.74$ , Fig. 2A).

For the radiotherapy group, there was a tendency towards the p73 GC/GC patients having better survival, although the difference was not significant ( $p=0.20$ , Fig. 2B).

Since there was a relationship between the p73 G4C14→A4T14 polymorphism and the expression of p53 and survivin in the radiotherapy group, a comparison between the patients who had p73 GC/GC genotype, negative p53 expression and weak survivin expression, with all other patients was done. In the non-radiotherapy group there was no significant difference in survival among the two groups ( $p=0.39$ , Fig. 3A). However, we found that the survival was significantly better in the group with GC/GC p73, negative p53 and weak survivin expression than all others, among those patients who received radiotherapy ( $p=0.03$ , Fig. 3B). The difference remained even after adjusting for both TNM stage and tumor differentiation in a multivariate analysis ( $p=0.01$ , RR, 7.63, 95% CI, 1.50-38.74). In further interaction analyses,

the correlations with prognostic significance of p73-p53-survivin was different between the patients without radiotherapy and with radiotherapy ( $p=0.04$ , RR, 6.37, 95% CI, 1.11-36.39).

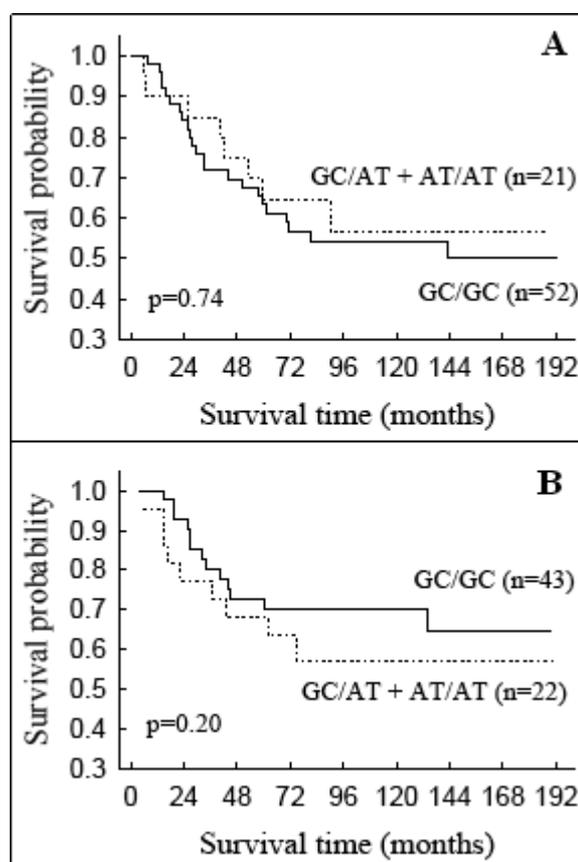
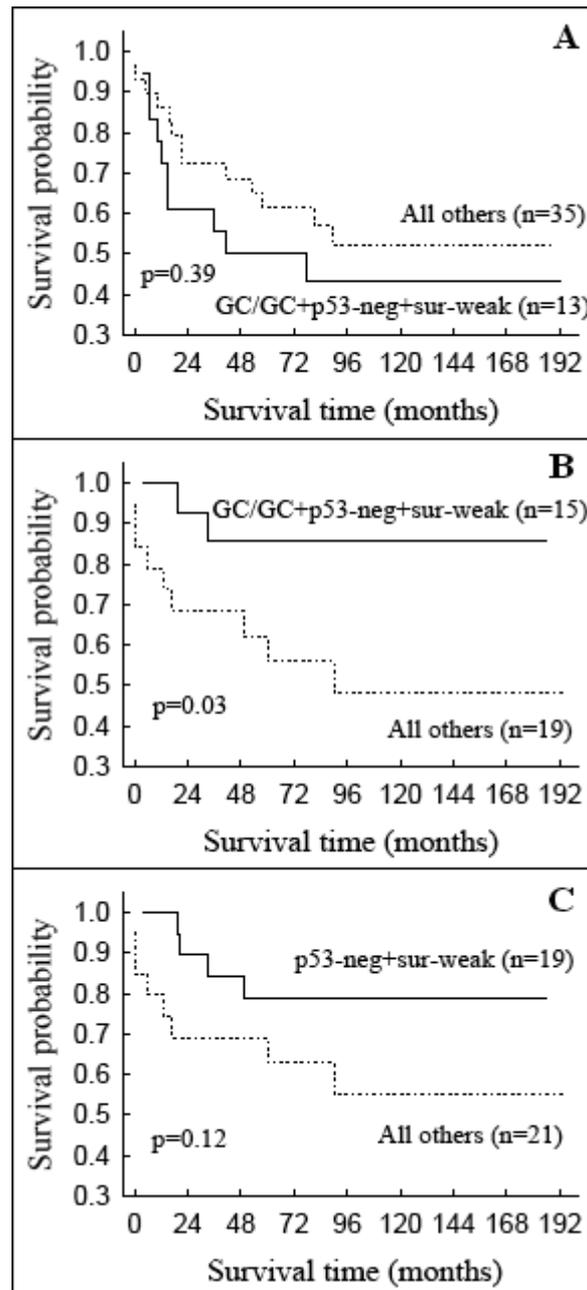


Fig. 2. Survival comparison of rectal cancer patients with either a GC/GC genotype with GC/AT+AT/AT genotype who were treated with surgery alone (A) or with preoperative radiotherapy and surgery (B).

To examine if the effect seen may come from the two other factors, p53 and survivin, a comparison of the patients who had both negative p53 and weak survivin expression, irrespective of p73 genotype, with all other patients in the radiotherapy group was done. The survival rate of the patients with negative p53 and weak survivin expression did not have a significant difference compared to all other patients ( $p=0.12$ , Fig. 3C). There was no survival significance for the three proteins in the non-radiotherapy group ( $p=0.37$ , data not shown).



*Fig. 3. Survival comparison of rectal cancer patients with a GC/GC genotype combined with negative p53 and weak survivin with all other patients who were treated with surgery alone (A) or who were treated with both preoperative radiotherapy and surgery (B). Survival comparison of rectal cancer patients having negative p53 and weak survivin with all other patients who were treated with preoperative radiotherapy and surgery, irrespective of the p73 genotypes (C).*

## Discussion

In the present study, we have shown that rectal cancer patients treated with preoperative radiotherapy and carrying the GC/GC genotype of p73 seem to survive longer, as compared to the patients with the GC/AT and AT/AT genotype. Also, the results show that patients in the radiotherapy group carrying the AT allele more often had a positive p53 and strong survivin expression. Furthermore, the combination of the GC/GC genotype of p73, with negative p53 and weak expression of survivin gave significantly better disease-free survival in patients who received preoperative radiotherapy, as compared to those with the other genotype/phenotype combinations. None of the three factors alone was related to survival in the patients with preoperative radiotherapy.

The role of the p73 G4C14→A4T14 polymorphism in relation to cancer risk has been investigated in populations with different genetic and social background and the results are inconsistent. Studies have shown that the AT/AT genotype or the AT allele is associated with a decreased risk of developing oesophageal cancer in an Irish population [16] and lung cancer in a Chinese population [11]. On the other hand, there are also studies indicating that the AT allele increases the risk of certain types of cancer, such as squamous cell carcinoma of the head and neck [12], cervical [14] and endometrial cancer in a Japanese population [15], and also lung cancer in a non-Hispanic white population [17]. We have earlier shown in a case-control study that the AT/AT genotype increases the risk of developing colorectal cancer [18]. Other studies have failed to find any correlation between the polymorphism and the risk of cancers [9, 11]. This discrepancy complicates an interpretation of the mechanisms behind differences seen between the genotypes of the G4C14→A4T14 polymorphism of p73.

The G4C14→A4T14 polymorphism does not cause an amino acid shift, but it occurs in a region of the transcript that could theoretically form a stem-loop structure, which

might alter the translational efficacy or affect the splicing of the transcript [3]. Regarding colorectal cancer, we have earlier shown that the GC/GC genotype is associated with worse survival as compared to both the GC/AT and AT/AT genotype. It has been shown in a number of studies that p73 is not a classical tumor suppressor, but is often overexpressed in tumors [22, 23].

Overexpression of p73 has been connected to a worse prognosis in colorectal cancer [8], and it has been speculated that the difference in survival seen between colorectal cancer patients with different genotypes was due to differences in p73 expression caused by the different alleles [18]. To further elucidate a possible relationship between the p73 polymorphism genotypes and p73 expression, we compared the genotypes of the p73 polymorphism with p73 protein expression on the same material [19] used in the present study. There was no significant relationship between the genotypes and the staining in the nucleus alone, cytoplasm alone or in both nucleus and cytoplasm. One reason for this non-relationship might be the small number of cases. The number of matched cases for the two studies were 114, where only a few cases (n=5) had the AT/AT genotype. Another reason may be that in our previous study [19], only total p73 expression was examined due to the lack of more specific antibodies at the time, while there are actually two isoforms of the p73 protein that may be relevant. The first p73 promoter creates the full length proapoptotic variant of the protein, called TAp73. The TAp73 isoform is homologous to p53, since it harbours the N-terminal transactivating (TA) domain, responsible for activating p53 responsive genes. The N-terminally truncated form,  $\Delta$ Np73, is a product of a promoter located in intron 3 [24]. The two isoforms regulate each other through a negative feedback loop, which also includes p53 [25, 26], where  $\Delta$ Np73 displays dominant negative behaviour toward TAp73 and wild type p53. In the current study, there was a tendency that the patients with the GC/GC genotype responded better to preoperative radiotherapy. The polymorphism is located

in exon 2, which contains the first promoter region of the p73 gene [3]. Certain genotypes may therefore alter the balance between the p73 isoforms, favoring the expression of a certain isoform possibly disturbing the otherwise tightly regulated balance between pro- and anti-apoptotic signals. To further investigate the impact of the p73 polymorphism genotypes in response to radiotherapy it would be of interest to compare the genotypes of the polymorphism with the expression of the two N-terminal isoforms of p73 proteins.

Since it seems that the p73 proteins play somewhat different roles in different tissues and different tumors, this may in part explain that different genotypes affect the development of tumors and outcome of treatment differently. In fact it seems that the p73 polymorphism and possibly the p73 protein play different roles in the colon and rectum. In this study we saw a tendency towards that rectal cancer patients treated with radiotherapy with the GC/GC genotype had better disease-free survival than those carrying the AT allele. In our previous study we saw the opposite, namely, that colorectal cancer patients carrying the AT allele had better survival [18]. The previous study was done on patients with cancer in either the colon or rectum as one group. With these discrepant results we went back to the previously studied material, divided the material into colon and rectum, and re-examined the relationship of the genotype with survival. It seems that in colon cancer the AT allele tended to be more favourable for overall survival, while in rectal cancer the GC allele tended to be more favourable (data not shown). However the differences did not reach statistical significance. Considering the embryological similarities of the colon and rectum the difference in survival seen with the different genotypes can not clearly be explained.

In this study we have seen a significant relationship in the radiotherapy group between the AT allele and the expression of p53 and survivin. A previous study on the rectal cancer material used in the present study showed the expression of p53 to be a significant predictive factor for treatment outcome after preoperative radiotherapy [20]. Survivin is an

anti-apoptotic protein that regulates the cell-cycle [27] and inhibits apoptosis, probably by inhibiting caspases 3 and 7 [28]. Survivin is undetectable or only weakly expressed in most normal adult tissues, but has been shown to be expressed in several types of tumors, including colorectal cancer [29]. Survivin increases radioresistance by inhibiting apoptosis and it has been shown that a high survivin expression is associated with a worse outcome in rectal cancer patients that have had preoperative radiotherapy [30, 31]. Transfection of rectal cancer cells with siRNA against survivin increases sensitivity to radiotherapy [30].

In the radiotherapy group, a significantly higher percentage of the patients with the AT allele had high expression of both p53 and survivin compared to the non-radiotherapy group. We further studied the relationship between the three proteins and saw that the combination of the p73 GC/GC genotype, with low expression of p53 and survivin, was related to better survival in the patients receiving radiotherapy. To look at p53 and survivin as predictive factors alone we compared the patients that had both low p53 and survivin expression to all other patients, regardless of the p73 genotype. Negative p53 and weak survivin expression were not significantly related to better disease-free survival, although a tendency towards longer survival was seen after radiotherapy in the patients with negative p53 and weak survivin. It seems that the combination of the p73, p53 and survivin or their interactions may play a further role in predicting prognosis in rectal cancer patients with radiotherapy.

In our previous study on the surgical material used in the present study no relationship was seen between p73 expression [19] and the expression of p53 [20] and survivin [21], however recently a possible relationship between p53, p73 and survivin has been seen [32-34]. Wild type p53, but not mutated p53 seems to repress survivin, either transcriptionally via E2F1 or via acetylation of the promoter region of survivin, containing two p53 binding elements [32, 34]. One study also looked at the regulation in the other

direction and saw that ectopic survivin also affects the levels of p53 in breast cancer cell lines, possibly through a survivin-dependent inhibition of caspases, in turn affecting mdm2 and the ubiquitination of p53. They also saw that forced expression of survivin increased the levels of both isoforms of p73, namely TAp73 and  $\Delta$ Np73 [33]. There seems to be a relationship between survivin and the p53 family proteins, but why then, is there an association between the high p53 and survivin expression and the AT-allele, and why in the radiotherapy-group?

Since a high p53 expression is associated with mutated p53, this would mean that patients with the AT allele, receiving radiotherapy, more frequently have mutated p53, than patients with the AT allele not receiving radiotherapy. It seems unlikely that either the radiotherapy or the p73 polymorphism could be related to p53 mutations, so we might need to consider the possibility that the results are coincidental. After all, the groups are relatively small. Regarding the survivin expression, it has been shown, using colorectal cancer cell lines, that radioresistant cell lines constitutively express higher levels of survivin, compared to a radiosensitive one, and that the survivin levels are further increased in the radioresistant cell lines after irradiation [31]. This could explain why the increase in survivin expression was seen in the radiotherapy group. Why it occurs among the patients carrying the AT allele, is unknown.

The results in the present study were obtained by genotyping specimens originating from either normal rectal mucosa or rectal cancer tissue. The p73 gene is very rarely mutated or changed in tumors [22], and when we previously compared the G4C14→A4T14 polymorphism genotype in DNA from 50 colorectal cancer patients with a heterozygous genotype in normal tissue and the matching tumor tissue, we showed that none of the cases had discrepant genotype in the normal and tumor tissue [18]. This indicates that the risk of showing mutational change rather than the polymorphism genotypes even from tumor tissue is relatively small.

In conclusion, our finding that the GC/GC genotype tends to be related to survival in rectal cancer patients with preoperative radiotherapy shows that the p73 G4C14→A4T14 polymorphism might be a factor influencing the response to preoperative radiotherapy. This effect was significant in combination with the previously known predictive factors wild type p53 and low levels of survivin.

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## Conflict of Interest Statement

To the best of our knowledge, there is no conflict of interest.