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Genetic variants in the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and death receptor (DR4) genes contribute to susceptibility to colorectal cancer in pakistani population

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Abstract

TRAIL mediated signaling in cancer cells has emerged as one amongst the most deeply studied molecular phenomenon. Data obtained through genetic studies has highlighted highly polymorphic nature of DR4 and in accordance with this concept, we aimed to investigate the association between Colorectal cancer and polymorphisms in TRAIL and DR4 gene. We selected 100 patients with colorectal cancer and 100 healthy, sex and age matched volunteers randomly. C626G and A1322G in DR4 gene were analyzed using Polymerase Change Reaction (PCR) – Restriction Fragment Length Polymorphism (RFLP) and Amplification Refractory Mutation System (ARMS) techniques. PCR-RFLP was used to study TRAIL 1595 C>T. TRAIL gene 1595 C>T genotypes percentage in colorectal cancer patients was statistically non-significant. CC was 43% in patients and 50% in controls. CT was 45% in patients and 43% in controls. TT was 12% in patients and 7% in controls. C allele was 0.655% in cancer patients and 0.715% in controls. T allele was 0.345% in patients and 0.285% in controls. DR4 gene 626 C>G genotypes percentage analysis indicated that CC was 28% in patients and 2% in controls. GC was 42% in patients and 40% in controls. GG was 30% in patients and 58% in controls. CC was statistically significant (p=0.00000207) in cancer patients. C allele was 0.49% in patients and 0.22% in controls. G allele was 0.51% in patients and 0.78% in controls. For DR4 A1322G, homozygous GG genotype was 36% in the patients and in controls. There was statistically insignificant difference (p> 0.05). The heterozygous GT genotype was 30% in patients and 29% in controls. This difference was statistically insignificant (p value > 0.05). Similarly, the homozygous genotype TT of the minor allele was (35%) in controls and patients (34 %). This difference was also statistically insignificant (p value > 0.05). C allele was 0.51% in patients and 0.5% in controls. T allele was 0.49% in patients and 0.495% in controls. Future studies must converge on a larger sample size, sporadic mutations of DR4 and TRAIL and expression profiling.

Key words: TRAIL, DR4, Cancer, Signaling, Apoptosis.

Introduction

Colorectal Cancer is a multifaceted and genomically complex disease as evidenced by rapidly accumulating preclinical and clinical studies. Increasingly it is being realized that genetic/epigenetic mutations, inactivation of tumor suppressors, overexpression of oncogenes and loss of apoptotic cell death are some of the widely studied biochemical mechanisms (1,2). Apoptotic cell death is doubtlessly a deeply studied mechanism in cancer and research over the years has gradually provided ever-increasing list of versatile regulators involved in apoptotic cell death. TRAIL, FasL and TNFα have been shown to transduce signals intracellularly, by their respective receptors. TRAIL is a ligand reported to induce apoptosis in cancer cells while leaving normal cells intact (3,4). TRAIL signals through particular receptors known as Death receptors (DR4 or DR5) (5,6). DRs have Death Domain (DD) through which DD containing proteins (FADD) interact. Death-inducing signaling complex (DISC) is a signalosome formed by assembly of FAS-associated death domain (FADD), pro-caspase-8 and death receptor to activate apoptosis-initiating caspase-8. Characteristically two different pathways have been noted to be functional in cancer cells to induce apoptosis. Extrinsic pathway is functionalized by ligand induced death receptor activation. Functionally active caspase-8 activates its downstream effector caspase-3. Intrinsically regulated pathway operates through release of cytochrome c from mitochondrion. Mitochondrial membrane permeability is enhanced via shuttling of truncated version of Bid into mitochondrion (7,8). Overwhelmingly increasing scientific evidence has considerably broadened our understanding of TRAIL resistant cancer phenotype. Mechanistically it has been shown that downregulation of DR4/DR5, genetic and epigenetic mutations notably impair TRAIL induced apoptosis in cancer cells.

Genetic studies have provided sufficient evidence of polymorphic nature of DR4 gene, but the most frequently noted polymorphism C626G is present within DR4 ectodomain. Contemporary studies provide divergent pieces of evidence related to intra/inter population variability related to DR4 polymorphisms including G422A, A683C, and A1322G.

Materials and methods

Sampling and DNA extraction

We selected 100 patients with colorectal cancer and

Table 1. Clinical parameters of colorectal cancer patients.

Clinical Parameters		Respective values and incidence percentage
Age		**Mean = 44.34 ± Std. Dev. 14.640**
Age group	Above 35	70%
	Below 35	30%
Marital status	Married	88%
	Unmarried	12%
Gender	Male	64%
	Female	36%
Type	Colon	52%
	Rectum	48%
Histopathology	Moderately differentiated	64%
	Well differentiated	14 %
	Poorly differentiated	22 %
Stage	Stage 1	4 %
	Stage 2	33 %
	Stage 3	43 %
	Stage 4	20 %

100 healthy, sex and age matched volunteers randomly. 88 percent of the patients were married and 12 percent were unmarried. It was seen that colorectal cancer was more prevalent in males (64%) as compared to females (36%). Histopathologically, moderately differentiated cases were 63.6%, poorly differentiated were 21.6% and well differentiated cases were 14.8%. Most frequently registered stages were stage 3 (43.2%) and stage 2 (33%). Stage 4 frequency was 20.5 % and stage 1 was 3.4% in colorectal cancer patients (Table 1). We used organic method to extract DNA from blood.

PCR-RFLP Methods for TRAIL 1595C/T Polymorphism

The primers for polymerase chain reaction (PCR) for this region were: Forward: 5′-TGA GCA CTA CAG CAA ACA TGA-3′ and reverse primer 5′-GCA CCA CTA AAA GAT CGC AGT-3′. The 391-base pair PCR product was digested by restriction enzyme RsaI at 37°C. After enzyme digestion, homozygous individuals for C allele (CC genotype) were identified by the presence of 59 and 332bp. The homozygous individuals for the T allele (TT genotype) were identified by the presence of 59, 146, 186 bp fragments. Digested products were visualized under UV light after running on 2% agarose gel electrophoresis.

PCR-RFLP Methods for DR4 C626G Polymorphism

The primers for polymerase chain reaction (PCR) for this region were: Forward: 5′-TGA GCA CTA CAG CAA ACA TGA-3′ and reverse primer 5′-GCA CCA CTA AAA GAT CGC AGT-3′. The 391-base pair PCR product was digested by restriction enzyme RsaI at 37°C. After enzyme digestion, homozygous individuals for C allele (CC genotype) were identified by the presence of 59 and 332bp. The homozygous individuals for the T allele (TT genotype) were identified by the presence of 59, 146, 186 bp fragments. Digested products were visualized under UV light after running on 2% agarose gel electrophoresis.

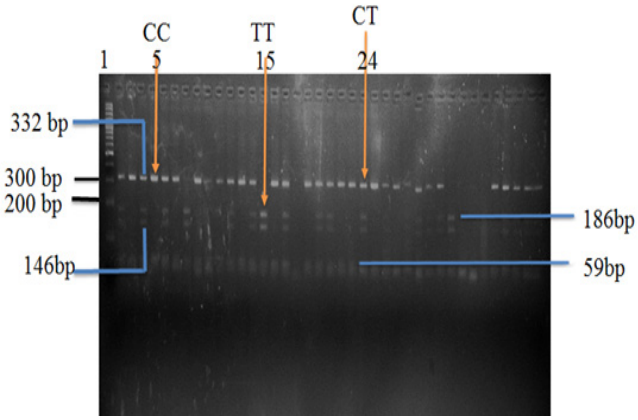

Figure 1. PCR-RFLP gel picture of TRAIL 1595 C/T genotype, Lane 1 showing 100 base pair ladder, Lane 5 showing homozygous CC, Lane 15 showing homozygous TT, Lane 20 and 24 showing heterozygous CT. Homozygous individuals for C allele (CC genotype) were identified by the presence of 59 and 332bp. The homozygous individuals for the T allele (TT genotype) were identified by the presence of 59, 146, 186 bp fragments.

PCR-ARMS Method for DR4 A1322G Polymorphism

Procedure used was mainly derived from the paper reported previously by Taştemir-Korkmaz et al. (9). However slight modifications were introduced. PCR conditions for A1322G polymorphism were 95 °C for 5 min, followed by 95 °C for 45 seconds, 53°C for 45 seconds and 72 °C for 45 seconds (35 cycles). The PCR mixture (25 µl) included 1X PCR Buffer (2.5µl), 25 mM MgCl2 (1.2µl), 2 mM dNTPs (1µL), primers (2µl), 5µl DNA, and 0.2U Taq Polymerase (Fermantas). Two different tubes were prepared for this purpose. Forward primer was added to the two tubes, but the reverse primers (ended with C and T) added separately. Products had 254 bp. We used internal control (CYP19 (Cytochrome P450, subfamily XIX) gene previously reported by Taştemir-Korkmaz et al. (9), forward primer: 5′-ATC TGT ACT GTA CAG CAC C-3′, reverse primer: 5′-CTC CAA GTC CTC ATT TGC T-3′.

Results

In the present study, 70 percent of the patients were above 35 years and 30 percent of the patients were below 35 years. 88 percent of the patients were married and 12 percent were unmarried. It was seen that colorectal cancer was more prevalent in males (64%) as compared to females (36%). Histopathologically, moderately differentiated cases were 64%, poorly differentiated were 22% and well differentiated cases were 14%. Most frequently registered stages were stage 3 (43%) and stage 2 (33%). Stage 4 frequency was 20% and stage 1 was 4% in colorectal cancer patients (Table 1).

TRAIL gene 1595 C>T genotypes percentage in colorectal cancer patients and control showed in table 2. There was no statistically significant role of homozygous CC or TT in colorectal cancer. CC was 43% in patients and 50% in controls. CT was 45% in patients and 43% in controls. TT was 12% in patients and 7% in controls. C allele was 0.655% in cancer patients and 0.715% in controls. T allele was 0.345% in patients and 0.285% in controls (Figure 1).

DR4 gene 626 C>G genotypes percentage in colorectal cancer patients showed in table 3. CC was 28%

Table 2. shows TRAIL gene 1595 C>T genotypes percentage in colorectal cancer patients.

Group studied	TRAIL gene 1595 C>T genotypes percentage			Allele frequency	
	CC	CT	TT	C	T
Patient (N=100)	43	45	12	0.655	0.345
Control(N=100)	50	43	7	0.715	0.285
p-value	0.467	0.832	0.251	0.956	0.938

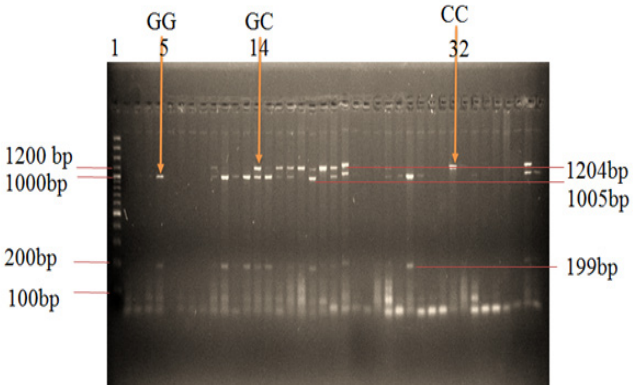

Figure 2. DR4 C626G genotype. Lane 1 is 100bp ladder. Lane 5 contained GG as evidenced by digested products for GG(1005+199). Lane 14 contained GC (1204+1005+199) and Lane 32 contained CC (1204).

in patients and 2% in controls. GC was 42% in patients and 40% in controls. GG was 30% in patients and 58% in controls. CC was statistically significant in cancer patients (p=0.00000207). C allele was 0.49% in patients and 0.22% in controls. G allele was 0.51% in patients and 0.78% in controls (Figure 2).

The distribution of DR4 A1322G genotypes are shown in table 4a and 4b. The homozygous GG genotype of the major allele was 36% in the patients and in controls. There was statistically insignificant difference (p> 0.05). The heterozygous GT genotype was 30% in patients and 29% in controls. This difference was statistically insignificant (p value > 0.05). Similarly, the homozygous genotype TT of the minor allele was (35%) in controls and patients (34 %). This difference was also statistically insignificant (p value > 0.05). C allele was 0.51% in patients and 0.5 in controls. T allele was 0.49 in patients and 0.495 in controls (Figure 3).

Discussion

In a recently published research addressing the relationship of DR4 and cancer progression, increased frequency of the DR4 C626G CC genotype in laryngeal squamous cell carcinoma patients than in controls was noted (10). Contrarily, no association was noted between DR4 gene polymorphism and lung cancer in Turkish population (9). Interestingly, in our population, colorectal cancer patients had significantly higher percentage of CC genotype (p<0.05). In our study, DR4 gene 626 C>G genotypes percentage analysis indicated that CC was 28% in patients and 2% in controls,whereas GC was 42% in patients and 40% in controls and,GG was 30% in patients and 58% in controls. CC was statistically significant (p<0.00001) in cancer patients. The frequency of C allele was 0.49% in patients and 0.22% in controls and, G allele was 0.51% in patients and 0.78% in controls. For DR4 A1322G, homozygous GG genotype was 36% in the patients and in controls. There was statistically insignificant difference (p> 0.05). The heterozygous GT genotype was 30% in patients and 29% in controls. This difference was statistically insignificant (p value > 0.05). Similarly, the homozygous genotype TT of the minor allele was (35%) in controls and patients (34 %). This difference was also statistically insignificant (p value > 0.05). G allele was 0.51% in patients and 0.5% in controls. DR4 Ala228Glu C > A and DR4 Thr209Arg C > G polymorphisms have previously not been noted to be connected with breast cancer risk (11). Similar findings

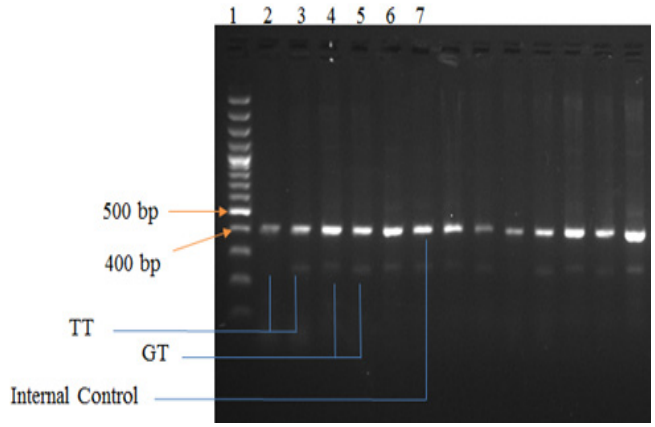

Figure 3. ARMS-PCR gel picture of DR4 A1322G, Lane 1 showing 100 base pair ladder. Lane 2 and 3 showing TT. Lane 4 and 5 show GT. Upper layer of bands is internal control and lower series of bands represent genotypes

Table 3. shows DR4 gene 626 C>G genotypes percentage in colorectal cancer patients.

Group studied	DR4 gene 626 C>G genotypes percentage			Allele frequency	
	CC	GC	GG	C	G
Patient (N=100)	28	42	30	0.49	0.51
Control (N=100)	2	40	58	0.22	0.78
***p*-value**	0.00000207 (<0.05)	0.82	0.0028 (<0.05)	0.74	0.811

Table 4. (a) and (b) shows analysis of DR4 A1322G.

Group	DR4 A1322G genotypes percentage		
	GG	GT	TT
Patient (N=100)	36%	30%	34%
Control (N=100)	36%	29%	35%
p-value of patients and controls	1	0.89	0.9

Group	Allele frequency	
	G	T
Patient (N=100)	0.51	0.49
Control (N=100)	0.5	0.495
p-value of patients and controls	1	1

had been reported in lung cancer patients of Russia (12). Thr209Arg polymorphism in the extracellular domain of DR4 was not associated with gastric cancer development and progression (13). Neither Thr209Arg (626C>G) nor Glu228Ala (683A>C) were reported to correlate individually with breast cancer risk. However, 626C-683C haplotype carriers had 3.5-fold higher risk (14). Thr209Arg was noted to exert protective effect in reducing rectal cancer risk and significantly increased risk was observed in Ala (228) carriers having advanced stages of colorectal cancer. However, 626C-683C haplotype carriers had 2.4-fold higher risk (15). DR4 exon 4 G/G genotype was associated with an overall decreased risk of bladder cancer in Caucasians (16). A1322G Polymorphism was identified in DD and reported to make the receptor less sensitive to TRAIL. G422A alteration causes an amino acid change (arginine to histidine) just 5′ to the ligand-binding domain of DR4 (17).

TRAIL C1595T and G1525A polymorphisms have previously been reported to be significantly linked with gastric cancer susceptibility in Chinese Han population (18). In our study of TRAIL 1595 C>T genotypes, there was no statistically significant role of homozygous CC or TT in colorectal cancer. CC was 43% in patients and 50% in controls, whereas CT was 45% in patients and 43% in controls, and TT was 12% in patients and 7% in controls. The frequency of C allele was 0.655% in cancer patients and 0.715% in controls, T allele was 0.345% in patients and 0.285% in controls. We did not find any correlation between TRAIL polymorphism and colorectal cancer susceptibility in our cancer patients.

Future studies must converge on expression analysis of DR4 in colorectal cancer patients. This will be helpful in identification of responders, poor responders and non-responders to different chemotherapeutic drugs.

Other articles in this theme issue include references (19-30).

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