MDR1 (C3435T) POLYMORPHISM IN PATIENTS WITH RENAL CARCINOMA

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Abstract

Introduction and aims: Human multidrug-resistant gene (MDR1) encode transmembrane P-glycoprotein, which plays important role in protection organism before various xenobiotics and carcinogens. MDR1 polymorphism may be associated with increased risk of development of renal carcinoma (RCC). The aim of our study was to evaluate MDR1 polymorphism in patients with RCC and compare various genotypes in relation to patological characteritics of tumors.

Material and methods: 94 patients (44 men, 50 women, median: 61 years, range: 27-83 years) with renal carcinoma (74 clear cell RCC, 20 non-clear cell RCC) were enroled in the study. We specified genotypes - single nucleotid polymorphism (SNP) in MDR1 gene (C3435T) in these patients by using polymerase chain reaction (PCR-RFLP).

Results: The results of our study observed no singinficant association between genotypes and allele frequencies by comparison patients with clear cell and non-clear cell RCC [CC vs. CT+TT: p=0,550, OR= 1,690, 95% CI 0,442-6,465 (Fisher´s exact test), C vs. T allele: p=0,603, OR= 1,207, 95% CI 0,593-2,457 (χ2 –test)]. There was also no singnificant association between genotypes and TNM stage of disease or histological grade of tumor.

Conclusions: Future studies are needed to compare the relation between MDR1 polymorphism and other histopathological characteristics of tumors and risk to renal cancer.

Introduction

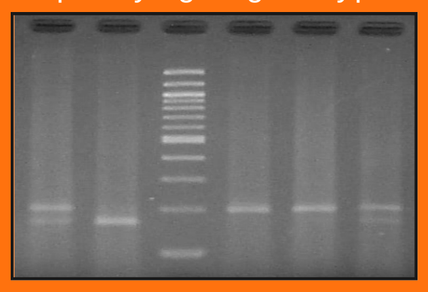
P-glycoprotein PGP

- transmembrane protein (ATP-dependent transporter) encoded by MDR1
- active efflux pump for a variety of toxins, carcinogens, drugs
- prevents intracellular accumulation of xenobiotics (e.g. in renal tubules)
- protects organism against exogenic and endogenic xenobiotics, toxins
- genetic variants of PGP can contribute to:
  - cancer susceptibility (modulate risk to cancer)
  - interindividual variability in therapeutic response

MDR1 polymorphism

- silent single nucleotide polymorphism (SNP) in MDR1 gene (localized in the middle of exon 26) is the most widely studied polymorphism of MDR1
- there is substitution of cytosine for thymine – C3435T
- is associated with variant gene expression and altered PGP function
- variant T allele is associated with increased risk to cancer

MDR1 (C3435T) polymorphism
PCR-RFLP – based assay
Specifying of genotypes



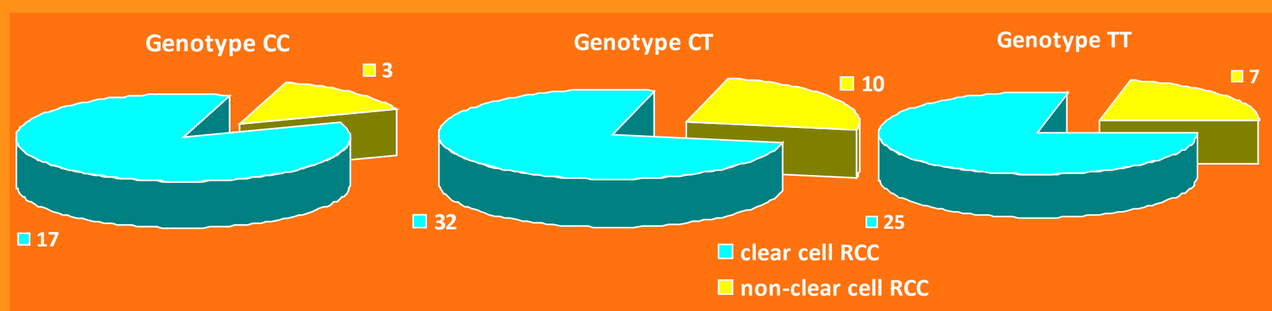
M - marker (100 bp ladder)

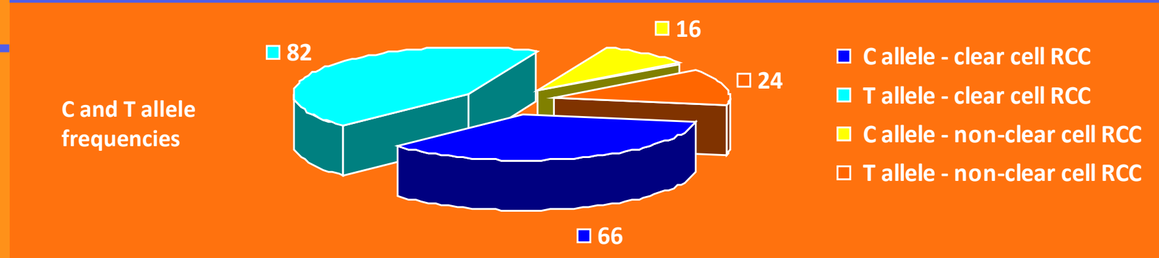
Material

- 94 patients with RCC hospitalized since 2011 to 2012 were enroled in the study
- 44 men and 50 women
- age 27 - 83 years (median: 61 y)
- we analyzed data of 94 patients with pathology-confirmed diagnosis of renal cancer:
  - 74 cler cell RCC
  - 20 non-clear cell RCC reffered to as:
    - papillary RCC (n=12)
    - unclasiffied RCC (n=3)
    - chromophobe RCC (n=2)
    - oncocytic papillary RCC (n=1)
    - oncocytic RCC (n=1)
    - oncocytoma (n=1)

Method

- constitutional DNA was isolated from patient´s blood samples
- genotyping of SNP in MDR1 gene was carried out by using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism)
- we determined 3 types of genotype (homozygous CC or TT, heterozygous CT genotype) and allele frequencies (C and T allele) in patients with RCC
- for all statistical analyses SPSS software (version 16.0) was used (the differences in genotypes and allele frequencies were determined using χ2 –test or Fisher´s exact test)





Results

- frequencies of genotypes and allelotypes were:
  - 21,3% (20) for CC, 44,7% (42) for CT and 34,0% (32) for TT genotype
  - 43,6% (82) for C allele and 56,4% (106) for T allele frequency
- we stratified data according to histopatological characteristics:
  - clear cell vs. non-clear cell RCC
  - low (T1+T2) vs. high (T3+T4) T stage of tumor
  - negative vs. positive N and M stage of tumor
  - low (G1+G2) vs. high (G3+G4) grade of tumor
- we observed no significant association between patients with clear cell and non-clear cell RCC in genotype and allele frequencies
  - CC vs. CT+TT: p=0,550, OR= 1,690, 95% CI 0,442-6,465 (Fisher´s exact test)
  - C vs. T allele: p=0,603, OR= 1,207, 95% CI 0,593-2,457 (χ2 –test)
- the data showed moderate higher frequency of CT+TT genotype and T allele in group with non-clear cell RCC, but it wasn´t statistically significant
- no singnificant differences were found across other histopathological characteristics of tumors:
  - low (T1+T2) vs. high (T3+T4) T stage of tumor:
    CC vs. CT+TT: p=0,723, C vs. T allele: p=0,920
  - low (G1+G2) vs. high (G3+G4) grade of tumor:
    CC vs. CT+TT: p=0,421, C vs. T allele: p=0,603
  - negative vs. positive N or M stage of disease:
    CC vs. CT+TT: p=0,451, C vs. T allele: p=0,764

		Genotypes			Alleles		Comparison of frequencies of genotypes or allele	
							CC versus CT+TT	C versus T
Variable	Patients (n=94)	CC (n=20)	CT (n=42)	TT (n=32)	C (n=82)	T (n=106)	P-value OR (95% CI)	P-value OR (95% CI)
Histology							clear vs non-clear RCC	clear vs non-clear RCC
clear cell RCC	(n=74)	17	32	25	66	82	0,550[1]*	0,603[2]*
non-clear cell RCC	(n=20)	3	10	7	16	24	1,690 (0,442-6,465)	1,207 (0,593-2,457)
T stage								
T1	(n=57)	11	25	21	47	67	T1+T2 vs T3+T4	T1+T2 vs T3+T4
T2	(n=11)	3	6	2	12	10	0,723[2]*	0,920[2]*
T3	(n=21)	5	8	8	18	24	0,821 (0,276-2,442)	0,975 (0,507-1,874)
T4	(n=4)	1	2	1	4	4		
undetermined	(n=1)	0	1	0	1	1		
N stage								
negative	(n=80)	17	35	28	69	91	negative vs positive	negative vs positive
positive	(n=13)	3	6	4	12	14	1,0[1]*	0,777[2]*
undetermined	(n=1)	0	1	0	1	1	0,899 (0,222-3,637)	0,884 (0,384-2,033)
M stage								
negative	(n=76)	15	35	26	65	87	negative vs positive	negative vs positive
positive	(n=17)	5	6	6	16	18	0,513[1]*	0,646[2]*
undetermined	(n=1)	0	1	0	1	1	0,590 (0,180-1,933)	0,840 (0,398-1,772)
N or M stage								
negative	(n=71)	14	33	24	61	81	negative vs positive	negative vs positive
positive	(n=22)	6	8	8	20	24	0,451[2]*	0,764[2]*
undetermined	(n=1)	0	1	0	1	1	0,655 (0,217-1,978)	0,903 (0,457-1,784)
Grade								
G1	(n=13)	1	6	6	8	18	G1+G2 vs G3+G4	G1+G2 vs G3+G4
G2	(n=43)	13	16	14	42	44	0,421[1]*	0,603[2]*
G3	(n=23)	4	10	9	18	28	1,80 (0,582-5,571)	1,178 (0,632-2,196)
G4	(n=9)	1	6	2	8	10		
undetermined	(n=6)	1	4	1	6	6		

Abbrevations: [1] Fisher´s exact test, [2] χ2 –test, * non-significant association

Conclusions

- genotypic variations has a key role in variability such as cancer susceptibility
- our study confirmed no significant association between all observed histo-pathological characteristics of tumors differentiated according to MDR1 genotypes and allelotypes (table)
- our results aren´t consistent with the hypothesis and other studies that the T allele and TT genotype is a risk factor for non-clear cell RCC
- there was also no singnificant association between allele frequency and TNM stage of disease and histological grade of tumor
- in the future we will focused on comparison MDR1 polymorphism in RCC patients with control group to determined cancer susceptibility
- future studies are needed to compare the relation between MDR1 polymorphism and other histopathological characteritics of tumors and risk to cancer