PCR in Cases of Prostate Cancer

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Introduction

The prostate cancer (PC) is one of the most frequent neoplasia in men even though not the deadliest. Every year more than 1,1 million men are diagnose with PC worldwide. The principal risks factors are: age, diet, familiar predisposition, race and hormones.

Gleason classification is use to graduate PC based upon the histologic pattern of tumoral growth. Additionally to this classification is use TNM that allow to describe the cancer severity for each individual.

Prostate-specific antigen (PSA) has been used as biomarker of PC, however it isn´t a specific marker for cancer detection. This protein is coded by KLK3 gene (located at 19q13.41) and it is regulated by androgen receptors through ligation to consensus sequences in promoter region of KLK3 – Androgen Response Elements (ARE). In ARE-I, located at -158 position, there is a single polymorphism nucleotide (SNP) named rs266882, which consists of substitution of guanine (G) for adenine (A).

Methods

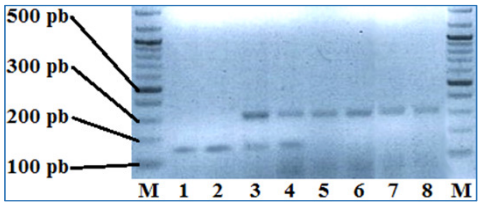
This study was realized in CiiEM Molecular Biology Laboratory, with samples from Pathology Service of Centro Hospitalar Barreiro-Montijo. We used samples from individuals diagnosed with PC, normal and tumoral tissue (70) and with benign prostatic hyperplasia - BPH (39).
DNA was extracted from paraffin embedded tissue sections (10x PCR buffer, proteinase K and ultra-pure water) and performed PCR-RFLP analysis of rs266882 polymorphism.
PCR primers are present in table 1 with the following conditions: 94˚C for 10 min; then 35 cycles at 94˚C for 1min, 59˚C for 1 min, and 72˚C for 40 sec and a final cycle at 72˚C for 10 min.

The restriction fragment of *KLK3* gene was obtain with *NheI* enzyme (2h at 37ºC).

Primer	Sequence	Target DNA
ARE-I Forward	5' – TTG TAT GAA GAA TCG GGG ATC GT – 3'	*KLK3* gene
ARE-I Reverse	5' – TCC CCC AGG AGC CCT ATA AAA – 3'	

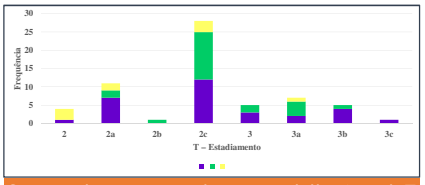
Results and Discussion

We obtained 66 amplifications of DNA fragment containing SNP rs266882 from PC samples (normal and tumoral tissue) and 36 from BPH samples. The fragment length was 300 bp. After enzymatic restriction we obtained the following band profile. Genotype was identical in tumoral and normal tissue in PC samples.

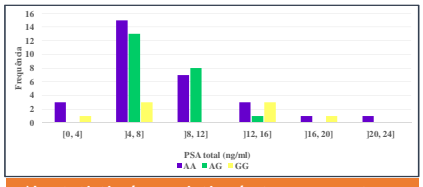


Genotype	PC	BPH	Total
AA	30 (48,4%)	18 (52,9%)	48 (50%)
AG	23 (37,1%)	12 (35,3%)	35 (36,5%)
GG	9 (14,5%)	4 (11,8%)	13 (13,5%)
Total	62	34	96

No statistical association (*p-value*=0,89, α=0,05)



Statistical association between alelle A and T stage (*p-value* = 0,046, α = 0,05, contingency coefficient 0,433)



No statistical association between genotype and total PSA levels (*p-value*=0,116, α=0,05)

	Prostatic Volume occupied by tumor
SNP	No statistical association (*p-value*=0,372)
Total PSA levels	Moderately positive correlation (0,348, *ρ* = 0,015, α=0,05)

Invasion Types	SNP	Total PSA levels
Vascular	No statistical association (*p-value* = 0,510)	No statistical association (*p-value* = 0,852)
Perineural	No statistical association (*p-value* = 0,215)	No statistical association (*p-value* = 0,252)
Capsular	No statistical association (*p-value* = 0,780)	No statistical association (*p-value* = 0,713)

Conclusion

This study didn´t revealed any association between rs266882 genotype and PC or BPH diagnosis. The presence of A allele at -158 position of KLK3 gene showed a statically significant association with T of stage tumor.
Total PSA level were associated with tumor volume in prostate.
The high incidence of PC worldwide makes the knowledge about subjacent molecular mechanisms very important to treat and diagnose the really aggressive tumors.