THE IMPACT OF IL-10 c. -592 C> A GENETIC VARIANT ON HPV E6/E7 mRNA EXPRESSION

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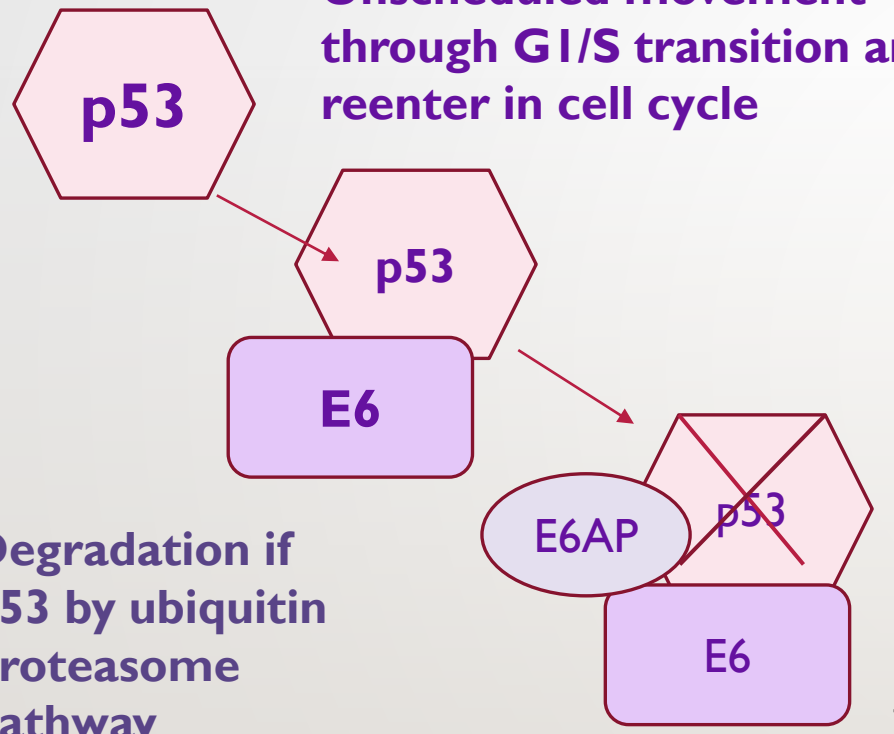
INTRODUCTION

- Cervical carcinogenesis is strongly associated with **persistent infection with high-risk (HR)** human papillomavirus (HPV), accompanied by HPV E6 and E7 messenger RNA (mRNA) expression as a hallmark of HPV persistence
- HPV E6 and E7 oncogenes trigger cervical cell carcinogenesis due to their interference with the steps of cervical **cell cycle**
- Hence, HPV E6/E7 mRNA testing is a **more specific test for HPV persistence** and a useful biomarker for predicting the condition that could undergo cell transformation.

MECHANISM OF HPV E6/E7 ONCOGENICITY

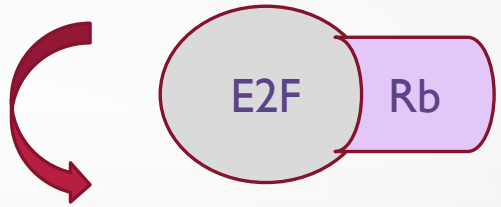
(INTERFERENCE WITH CERVICAL CELL CYCLE)



Unscheduled movement through G1/S transition and reenter in cell cycle

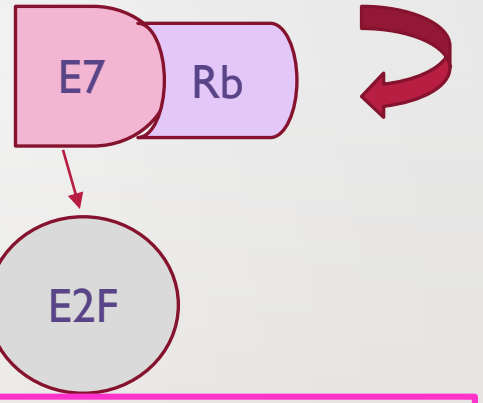
Degradation if p53 by ubiquitin proteasome pathway

Inhibition of apoptosis



Promotes off of the cycles

Promotes on of mitosis



-Normally prevents continuous replication and proliferation
- E7/pRb – complex release the E2F that promotes cell cycle activation

-The genome's gate-keeper
- Recognizes DNA damages
- Initiates protective response, and blocks the cell cycle
- Initiates apoptosis of damaged tissue
- E6 initiates ubiquitin-mediated degradation of p53

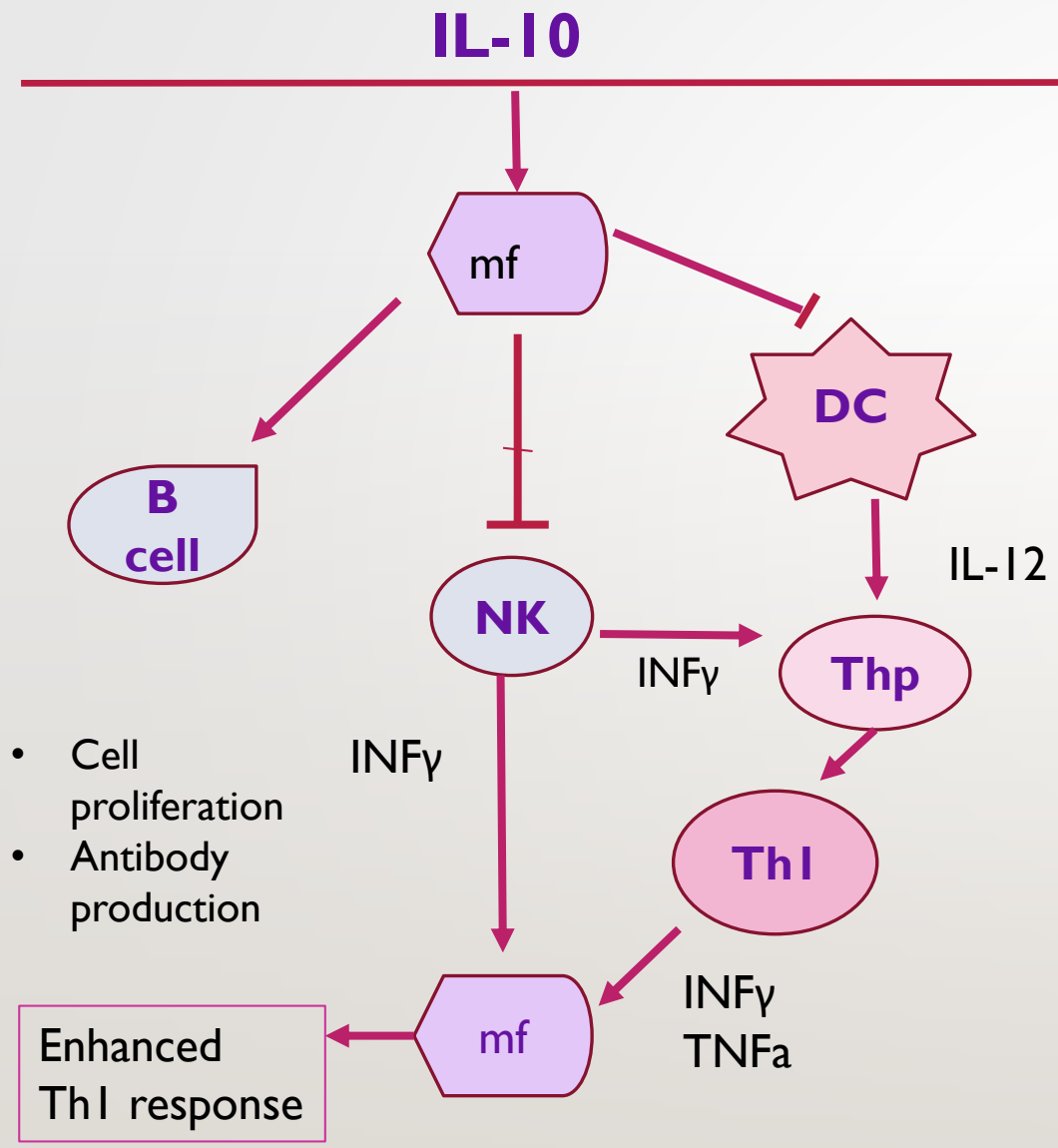
THE QUESTIONS THAT WE WILL FOCUS ON

- What immune factors influence VIRAL PERSISTENCE,
- How does IL-10 influence CONTINUING HIGHER EXPRESSION OF HPV E6/E7,
- weather IL-10 c. 592 C>A VARIANT INFLUENCE ON E6E7 EXPRESSION

IL-10 CYTOKINE AND HPV E6/E7 RELATION

- More than 80% of HPV infections spontaneously resolve in response to the host immunity and only a small percentage remains persistent.
- **WHY SOME HPV REMAIN PERSISTENT AND THE REST SPONTANEOUSLY RESOLVED**
- The **host's immunity** and its **host's genetics**, are the most important influencing factors
- Immunomodulators such as **cytokines are particularly important in viral clearance** or persistence
- **Th1 cytokines are proinflammatory** and they promote **viral clearance** while the Th2 cytokines promote a chronic state of the disease
- **Additionally, studies have found that their genetic variants can change the level of their expression and** are associated with **higher or lower protein expression**

MECHANISM OF IL-10 ACTIVITY IN THE IMMUNE RESPONSE OF VIRUSES



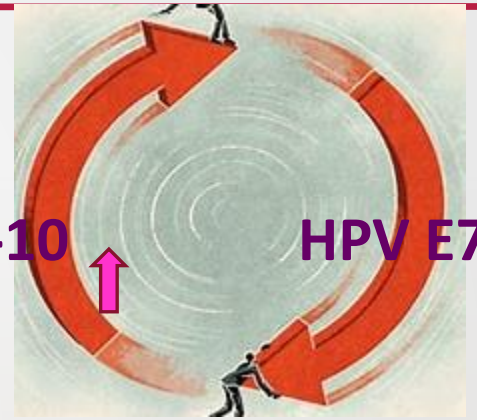
- Cell proliferation
- Antibody production

- Interleukin IL-10 belongs to Th2 profile
- It has an **immunosuppressive effect** that may influence **viral persistence** due to the inhibition of Th1 cytokine production, **supporting chronic viral disease**
- in the presence of HPV E6/E7 proteins, it has an anti-inflammatory function and, later it promotes tumor growth due to initiating higher E7 expression
- **The elevated IL-10 levels** inhibit Th1 cytokine production through:
  - direct targeting of immune effector types,
  - indirectly modulate immune function by preventing the maturation of macrophage and dendritic cells, thereby limiting the expression of co-stimulatory antigen presentation and chemokine secretion capacity of the host.

IL-10 CYTOKINE AND HPV E6/E7 MRNA RELATION

So how does it function in HPV infections ???

- IL-10 (Th2 cytokine) → immunosuppression and HPV E↑
- CC variant of IL-10 c. -592 → higher level IL-10 protein
- IL-10 protein↑ → HPV E7 expression ↑ → risk for carcinogenesis



vicious cycle

•Generally, IL-10 induces expression of E6/E7, and vice versa, HPV E2, E6, and E7 proteins **induce IL-10** expression, resulting in a **vicious cycle** that leads to infection progression to CIN or CCa.

•Studies have shown IL-10 production depends on its genetic variant, especially those in promoter genes that could be associated with higher or lower IL10 expression, and IL-10 c. -592 CC genotype and C allele variant located in the promoter of this gene, are associated with CIN lesions and CCa cases

• However, whether this genetic variant additionally influences the continued and higher expression of E6/E7 is still unknown

AIM

- **We conducted a study to find whether there is an association between IL-10 c. -592 C> A and HPV E6/E7 mRNA positivity in a group of Macedonian women and whether the finding could be an additional predictive marker for women susceptible to CCa development**

PATIENTS

- The study group consisted of 272 women aged 17–67 years, who underwent a routine cervical cancer screening program.
- Endocervical swabs collected by a gynecologist from the University Clinic for Gynecology and Obstetrics, Skopje were provided to the Laboratory for Virology and molecular diagnostics at the Institute of Public Health of the Republic of North Macedonia, Skopje.
- All the samples were submitted to both testing: HPV E6/E7 mRNA expression and detection of HPV DNA as well as genotyping for the IL-10 c.-592 C/A variant.
- The study group was divided into three subgroups according to the results from the HPV testing: a **positive group** with positive for both tests: HPV E6/E7 mRNA expression and HPV DNA (n = 108); a **negative group** - patients negative for the HPV E6/E7 mRNA expression but the positive for HPV DNA (n = 51); and a **control** group consisted of patients negative for both tests (n = 113), and no history of any previous cervical abnormality

METHODS

NUCLEIC ACID ISOLATION

Total nucleic acid was extracted from the pellet after centrifuging the cervical specimen preserved in PreservCyt/ThinPrep solution (Cytyc Corporation) or viral transport medium (phosphate-buffered saline) according to the NucliSens protocol using the miniMAG platform (bioMerieux).

HPV DNA DETECTION AND GENOTYPING: was done using Seeplex® HPV4A ACE screening, assay Seegene, Corea.

HPV mRNA detection HPV mRNA: was detected with the PreTect HPV Proofer® test according to the manufacturer's instructions.

IL-10 c. -592 C> A GENOTYPING: was analyzed using PCR-restriction fragment length polymorphism, and sequencing on ABI PRISM 310 Genetic Analyzer (Thermo Fisher Scientific)

STATISTICAL ANALYSIS:

The genotype distribution and allelic frequencies were analyzed using Pearson's $\chi 2$ test and Fisher's exact test, or its extension, considering $p \leq 0.05$ as a significance threshold. **The odds ratio (OR)** and the confidence interval (CI) of 95% by the Speckle image statistical analysis statistics method (https://www.quantitativeskills.com/sisa/ statistics/twoby2.htm) **were calculated to estimate the probability of association of the studied polymorphisms with positive HPV E6/E7 mRNA** expression. The ORs were performed for rs1800872 polymorphism under the allelic model (C vs. A), homozygous model (CC vs. AA), recessive genetic models (AA vs. CC + AC), heterozygous model (CC vs. AA), and dominant inheritance model (AA + AC vs. CC), respectively.

RESULTS

Genotype and allele distribution of c. -592 C> A variants in double positive, negative, and control groups

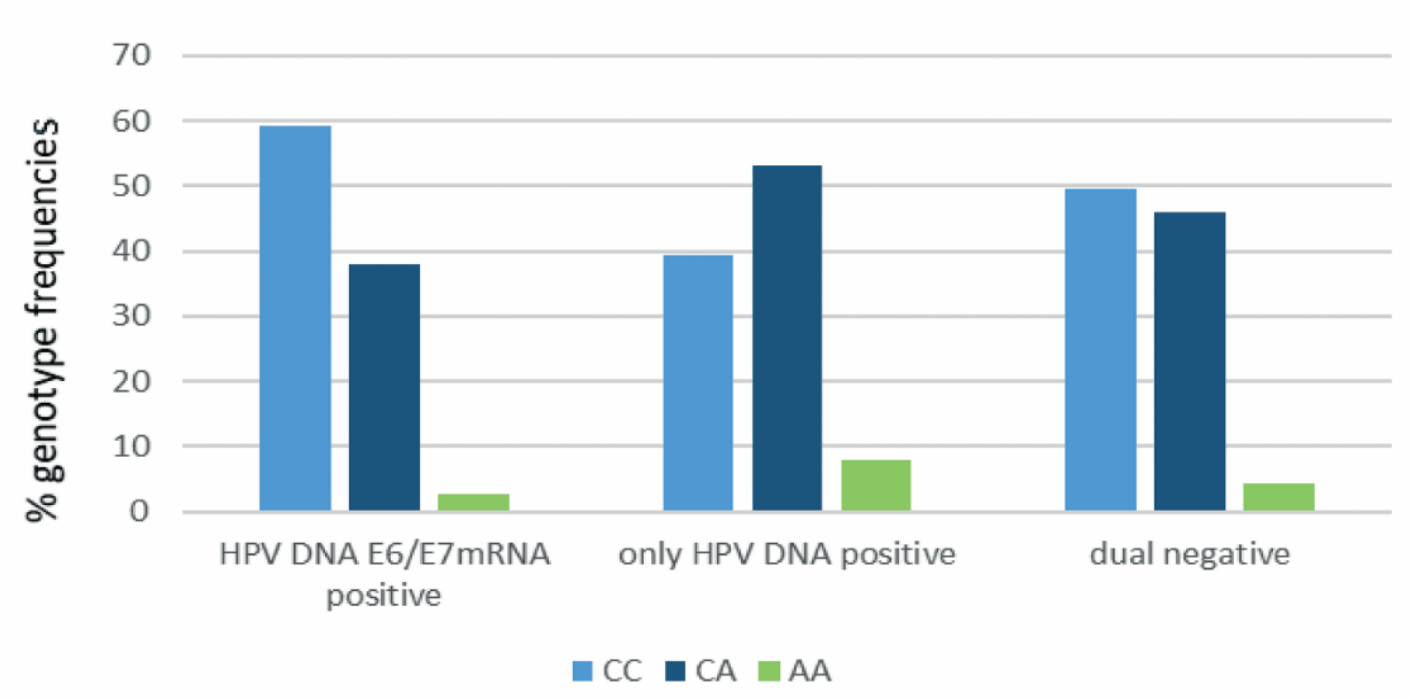
Groups	Genotype distribution (%)				Allele frequency (%)		
	CC n (%)	AC n (%)	AA n (%)	Total n (%)	C n (%)	A n (%)	Total n (%)
*positive	64 (59.3)	41 (38.0)	3 (2.7)	108	169 (78.2)	47 (21.8)	216
negative	20 (39.2)	27 (53.0)	4 (7.8)	51	67 (65.7)	35 (34.3)	102
control	56 (49.6)	52 (46.0)	5 (4.4)	113	164 (72.6)	62 (27.4)	226
total	140 (46.9)	120 (48.5)	12 (4.6)	272	400 (71.2)	144 (28.8)	544

*Positive: dual positive (HPV E6/E7 mRNA and HPV DNA positive)
*Negative: only HPV DNA positive

- CC genotype is significantly ($p = 0.018$) more common in the positive group (59.3%) compared to the negative group (39.2%), and compared to the negative and control together ($p = 0.04$), but not compared to the control group (49.6%) alone

- The frequency of the C allele was 78.2% in the positive group, 65.7% in the negative group, and 72.6% in the control group. The C allele was significantly more frequent in the positive group ($p = \mathbf{0.016}$) compared to the negative group and with borderline significance compared to the other groups ($p = 0.04$).

DISTRIBUTION OF GENOTYPE FREQUENCIES OF IL-10 c. -592 C>A IN THE THREE GROUPS



•The genetic background of the host strongly influences human response to infections and recently has been extensively explored to predict the infection outcome.

•A common c. -592 C> A was shown to be associated with susceptibility to various malignancies and with different infection outcomes.

STATISTICALLY SIGNIFICANCE OF IL-10 C. -592 C> A GENOTYPES FREQUENCY COMPARED AMONG THE THREE STRATIFIED GROUPS.

IL-10 c. -592	P1	OR	(95%CI)	P2	OR	(95%CI)	P3	OR	(95%CI)
C / A	0.016	1.88	1.11-3.16	0.04	1.5	1.01-2.25	0.166	1.36	0.88-2.1
CC /AC	0.034	2.1	1.05-4.24	0.058	1.62	0.98-2.68	0.18	1.45	0.84-2.49
CC /AA	0.05	4.27	0.88-20.69	0.016	2.53	0.66-9.73	0.38	1.90	0.44-8.33
CC/AA+AC	0.018	2.25	1.14-4.45	0.037	1.68	1.03-2.75	0.148	1.48	0.87-2.52
AC+CC/AA	0.15	2.98	0.64-13.84	0.289	2.03	0.54-7.68	0.51	1.62	0.38-6.95

P1= HPV dual positive (positive group) vs HPV DNA positive (negative group)
P2= HPV dual positive (positive group) vs HPV DNA positive (negative group) +control group
P3= HPV dual positive (positive group) vs control group

- *The limitation of the study is the small number of samples investigated*

- The CC in the positive group is associated with a higher rate of HPV E6/E7 mRNA expression **OR= 2.25** (95% CI: 1.14–4.45) compared to the negative group and OR= 1.68 (95% CI: 1.03–2.75) compared to the rest groups together

- the C allele is associated with the HPV E6/ E7 mRNA expression OR=1.88 (95% CI: 1.11–3.16) compared to the negative group; and OR = 1.5 (95% CI: 1.01–2.25) compared with both (negative and control group together), respectively.

- This OR was insignificant when the positive group was compared to the control group alone

CONCLUSION

- The CC/C variant of IL-10-592C/A SNP might be used as a **marker for predicting the higher** rate of HPV E6/E7 mRNA expression and **predicting HPV persistence**, **but only after the onset of infection, (thus it might be useful in cervical cancer risk assessment)**
- The CC genotype is not associated with susceptibility for HPV DNA onset, given the absence of association with HPV DNA alone.
- The limitation of the study is the small number of samples investigated; therefore, both findings warrant confirmation in wider epidemiological and molecular studies with more participants.
- The exact biological mechanism that underlines this association remains unknown