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REVIEW ARTICLE

Fighting against human papillomavirus: the 25-year old contribution of the University of Crete School of Medicine

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Summary

Twenty five years have passed since the first research efforts in Greece on human papillomavirus (HPV) performed by the Department of Clinical Virology at the University of Crete School of Medicine. HPV infection in the human cervix was initially evaluated in relation to the host mutational and transcriptional activation of the ras/raf genes pathway, p53 gene polymorphisms, neo-angiogenesis-related gene expression and G1/S phase transition. A series of epidemiological studies ensued, evaluating HPV infection in the ophthalmic pterygium, benign laryngeal tumors, parotid lesions, nasal polyposis, actinic keratosis, aborted material and non-genital cancers. The observed geographical variations of different HPV types within the Hellenic population indicated a higher prevalence of HPV 18 on the island of Crete compared to mainland Greece. Moreover, our research led to the investigation of the mother-to-infant HPV transmission via human breast milk and the detection of novel HPV types in juvenile recurrent respiratory papillomatosis. We also evaluated the presence of HPV in the respiratory tract of asymptomatic children and the relationship between maternal HPV infection and neonatal prematurity. Despite the introduction of the current prophylactic vaccines against HPV into clinical practice, HPV remains a challenging target for the next generation of researchers, as the war against HPV continues.

Key words: *adults, children,Greece, HPV, human papillomavirus, research, University of Crete*

Introduction

HPV, which comprises a remarkably diverse family of epitheliotropic double-stranded DNA viruses, has been the subject of intensive research, worldwide, for the last 35 years [1]. To date, the epidemiology, pathophysiology and molecular virology of HPV-associated clinical infections occurring in both children and adults have been clarified in detail, including skin warts, genital warts, juvenile recurrent respiratory papillomatosis (RRP), cervical squamous intraepithelial lesions and cervical cancer [2,3]. Recently, two major scientific events, the 2008 Nobel Prize awarded to Professor Harald zur Hausen, the 'Father of HPV Virology', who suggested the connection between HPV and cervical cancer and the introduction of the two prophylactic vaccines against HPV into clinical practice, provided a further expansion of the basic and clinical research on HPV.

The Department of Clinical Virology at the University of Crete School of Medicine in Heraklion in Crete, directed since 1990 by Professor Demetrios A. Spandidos [4], was the first research team in Greece, which developed the polymerase chain reaction (PCR) for HPV DNA detection in collaboration with the Institute of Biological Research and Biotechnology of the National Hellenic Research Foundation in Athens. Moreover, it was among the first in the European Union, which gave the possibility to women to be tested for HPV DNA almost 10 years before the international recommendations supporting HPV testing in women [5]. It also established the human cervix as an excellent educational model for medical undergraduate teaching. This model presenting human carcinogenesis induced by a viral cause was fondly used for the teaching of third-year medical

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students in the University of Crete in the undergraduate course of Clinical Virology. This course was included since 1984 in the basic undergraduate training schedule [6] of the University of Crete School of Medicine, which is the only Medical School in Greece teaching Clinical Virology as a separate course. As 25 years have passed since these first scientific efforts on HPV research in Greece and 20 years since the first report by Koffa et al. [7] among all relevant research articles on adults and children presented in Tables 1 [7-34] and 2 [35-42], we briefly present the contribution of the University of Crete School of Medicine to the basic and clinical pre-vaccination research on HPV.

HPV pre-vaccination research in adults

Since 1990, the Department of Clinical Virology at the University of Crete School of Medicine aimed to develop new protocols and ameliorate DNA extraction and PCR techniques using paraffin-embedded specimens, fresh biopsy material, as well Pap smear test samples (see review by Zaravinos et al. [43]). The primary question that needed to be answered was based on primary unpublished observations that HPV infection is a necessary but not sufficient condition for HPV-associated carcinogenesis, as in several cases HPV-associated squamous intraepithelial lesions (SILs) do not progress to cervical cancer, but regress. In this context, the presence of HPV DNA in the human cervix was evaluated in relation to the host cellular gene activation at the mutational and transcriptional level, including the expression and the mutational activation of the *ras/raf* genes pathway [7,13,14,20,22,24], *p53* gene polymorphisms [15,16], neo-angiogenesis-related gene expression

Table 1. The contribution of the Department of Clinical Virology at the University of Crete School of Medicine on HPV pre-vaccination research in adults

Year	*Authors [Ref]*	*Contribution*
1994	Koffa et al. [7]	Evaluation of HPV infection and ras genes mutations in cervical cancer
1995	Giannoudis et al. [8]	Detection of HPV infection in nasopharyngeal cancer
	Koffa et al. [9]	Detection of HPV infection in cervical SILs
	Koffa et al. [10]	Detection of HPV and HSV infection in cervical cancer
1996	Noutsou et al. [11]	Detection of HPV infection in lung cancer
1998	Sifakis et al. [12]	Absence of HPV infection in spontaneous abortions
	Dokianakis et al. [13]	Detection of HPV infection and ras genes mutations in cervical smears
1999	Dokianakis et al. [14]	Detection of HPV infection and ras genes mutations in cervical smears
2000	Dokianakis et al. [15]	Detection of HPV infection and p53 gene mutations in non-melanoma skin cancer
	Dokianakis et al. [16]	Detection of HPV infection and p53 gene mutations in cervical cancer
	Biliris et al. [17]	Detection of HPV infection in non-melanoma skin cancer
2001	Detorakis et al. [18]	Detection of HPV infection in ophthalmic pteryrium
	Sourvinos et al. [19]	Evaluation of HPV infection and p53 gene mutations in laryngeal cancer
2002	Prokopakis et al. [20]	Evaluation of HPV infection and ras genes mutations in cervical SILs
	Soulitzis et al. [21]	Evaluation of HPV infection and p53 gene mutations in bladder cancer
2004	Mammas et al. [22]	Evaluation of HPV infection and ras genes expression in cervical cancer
2005	Lyronis et al. [23]	Detection of HPV infection in oesophageal cancer
	Mammas et al. [24]	Evaluation of HPV infection and ras genes expression in cervical SILs
2007	Baritaki et al. [25]	Evaluation of HPV infection and VEGF and TGF-beta1 expression
	Vageli et al. [26]	Detection of HPV infection in parotid lesions
	Balis et al. [27]	Detection of HPV infection in prostate cancer
2008	Mammas et al. [28]	Detection of HPV geographic variations in Greece
	Lyronis et al. [29]	Evaluation of HPV infection and ras genes mutations in oesophageal cancer
	Arvanitis and Spandidos [30]	Evaluation of HPV infection and G1/S phase transition expression in cervical SILs and cervical cancer
2009	Zaravinos et al. [31]	Detection of HPV infection in nasal polyposis
2010	Zaravinos et al. [32]	Detection of HPV infection in actinic keratosis and non-melanoma skin cancer
2013	Panagiotakis et al. [33]	Detection of HPV, HSV and PyV infection in bladder cancer
2014	Sarchianaki et al. [34]	Detection of HPV infection in lung cancer

Table 2. The contribution of the Department of Clinical Virology at the University of Crete School of Medicine on HPV pre-vaccination research in children

Year	*Researchers [Ref]*	*Contribution*
2006	Mammas et al. [35]	Detection of HPV 16 and HPV 11 in tonsillar and adenoid tissues in children
2008	Mammas et al. [36]	Absence of HPV 16 and HPV 18 in benign skin lesions in children
2010	Mammas et al. [37]	Detection of HPV 13, HPV 39, HPV 40 and HPV 56 in juvenile RRP
	Mammas et al. [38]	Evaluation of maternal HPV infection and neonatal prematurity
	Mammas et al. [39]	Report of a case examining mother-to-infant HPV transmission via human breast milk
	Mammas et al. [40]	Detection of HPV 16, HPV 18 and HPV 31in bronchoalveolar lavage samples in children
2011	Mammas et al. [41]	Absence of HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56 and HPV 58 in human breast milk
2012	Mammas et al. [42]	Evaluation of oral HPV persistence in children and mode of delivery

[25] and G1/S phase transition [30]. These data provided valuable evidence supporting additional host cellular targets of both HPV E6 and E7 that play an important role in malignant cellular transformation(see review by Mammas et al. [44]).

These studies were followed by a series of epidemiological detection analyses of HPV DNA in non-genital cancers, including nasopharyngeal cancer [8], lung cancer [11,34], non-melanoma skin cancer [17], laryngeal cancer [19], bladder cancer [21,33], oesophageal cancer [23,29] and prostate cancer [27]. The presence of HPV DNA was also assessed in benign lesions, including aborted material [12], ophthalmic pterygium [18], benign laryngeal tumors [19], parotid lesions [26], nasal polyposis [31] and actinic keratosis [32]. Interestingly, almost 20 years after the initial work by Noutsou et al. [11], the presence of HPV DNA in lung cancer has been re-suggested in the literature as an aetiologic agent in lung carcinogenesis, with possible implications in the vaccination strategies against HPV [45-47]. HPV involvement in non-genital anatomical areas has been reviewed in detail in the recent reviews by Mammas et al. [48] and Radojicic et al. [49]. Despite the 'inconsistency' of these reports [1], further data collected during the vaccination period will provide the final answer of the observation that HPV exists in the progression of human carcinogenesis in multiple anatomical sites.

Another significant observation of the Department of Clinical Virology at the University of Crete School of Medicine was the presence of geographical variations of different HPV types within the Hellenic population [7,9,10,13,14,20,28]. This pre-vaccination data indicated a higher prevalence of HPV 18 on the island of Crete compared to mainland Greece (Athens, Larissa) and a significant trend for difference in the rates of non-HPV 16 or HPV 18 in women between the two areas. These observations are expected to have a significant impact on the effectiveness of the current vaccination programmes against HPV. The epidemiology of HPV infection in the Hellenic population definitely requires further evaluation with larger-scale epidemiological surveys under the collaboration of all 7 Medical Schools in Greece.

HPV pre-vaccination research in children

During the past decade, the Department of Clinical Virology at the University of Crete School of Medicine was one of the few research teams worldwide, which excessively studied HPV infection from the paediatric point of view (Table 2) [35-42]. This was mainly achieved by expanding the usage of molecular methodologies, such PCR techniques, in fresh and paraffin-embedded specimens collected from children after obtaining the relevant institutional and parental ethical approval. Our initial interest in HPV in children was inspired by the case of a 2-year-old child with juvenile RRP treated in the Paediatric Intensive Care Unit at the 'Aglaia Kyriakou' Children's Hospital in Athens. At that time, basic research on HPV in children in Greece was limited. The paediatric HPV story in childhood was so unpopular that in 2002 the project of the Ph.D. thesis that would support this research was initially rejected by the local Research Committee. In the following years, our research - summarized recently by Mammas and Spandidos [50] - led out to: A: the detection of high-risk HPV DNA in the upper and lower respiratory tract in children; B: the investigation of the presence of high-risk HPV DNA in skin lesions

in children; C: the evaluation of the mother-to-infant HPV transmission via human breast milk; D: the detection of novel HPV types, including HPV 13, HPV 39, HPV 40, HPV 56 in juvenile RRP; and E: the evaluation of HPV infection in relation to neonatal prematurity and the mode of delivery. To date, we have enthusiastically supported the introduction of the two current prophylactic vaccines against HPV in adolescent boys and the change of the current age group for vaccination against HPV from childhood to infancy [51,52]. We have also used for the first time in the literature the term 'Trojan horse oncogenic strategy' to describe the physical history of HPV in childhood [53]. Recently, we proposed the genomic diversity of HPV types, characterized by the intratypic variability of their genomic regions, as an additional marker for the success of the current vaccination programmes against HPV [54]. All these observations in childhood and adulthood require further investigation and re-evaluation during the vaccination period, which has already begun. HPV remains a challenging basic, epidemiological and clinical research target for the next generation of researchers. Indeed, the end of the war against HPV has not yet been reached.

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