

# Uterine Cervix Carcinoma: Recent Biological Data and Update for Improving Follow-Up and Treatment

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**KEY WORDS:** human papillomavirus (HPV), circulating tumor DNA (ctDNA), cervical cancer, gynecological oncology, oncogenes

IMAJ 2012; 14: 700-704

Cancer of the uterine cervix has two main characteristics: a) it is generally preceded by an intra-epithelial neoplasm of long duration, which could have been diagnosed by a simple Pap smear and treated by a simple conservative surgical procedure, and b) the majority of cervical carcinomas are associated with certain specific types of human papillomavirus, particularly types 16 and 18. Over the last 20 years, research has brought a new approach regarding diagnosis, prophylaxis and treatment of uterine cervical cancer. At present, biological research has designed a prophylactic vaccination to prevent intraepithelial neoplasias associated with HPV-16 and 18, precursors of about two-thirds of invasive cancers. Another vaccine targeting the viral proteins through specific cellular immunization has been tested on patients with established intraepithelial vulvar lesions and has resulted in regression, but is limited to intraepithelial lesions [1]. These results strengthen the current interest in the biological approach to cervical carcinomas. There is still one key question that has yet to be answered: which patients benefit from this treatment approach?

Genetic typification of the tumoral lesions has allowed us to design follow-up biological protocols which facilitate the diagnosis of relapse at the subclinical stage. The specific typification makes it possible to conceive a new therapeutic approach to cervical cancers in relapse by targeting specific tumoral cells in patients presenting a limited tumoral mass. In this review we describe and analyze the biological bases supporting such an approach.

## PREMATURE STAGES OF CERVICAL NEOPLASIAS

The natural history of cervical neoplasias is well known. It is commonly accepted that HPV infections without detect-

able lesions are frequent in young women and transitory in the great majority of cases. Persistence of the infection is the main risk factor for the development of intraepithelial neoplasia, which can be of high grade. A large proportion of intraepithelial neoplasias can regress spontaneously [2]. The nature of this regression underlines the major role of the immune system in controlling infections in the early stages of neoplastic lesions.

Concomitant human immunodeficiency virus and HPV infection increases both HPV persistence and the risk of invasive cervical cancer. A recent Israeli study by Leibenson et al. [3] proved that the prevalence of HPV carriage among HIV-positive woman and pathological cervical cytology were much higher than in the general population. An extremely high prevalence of pathological colposcopies requiring further treatment was also found in their group of patients. Screening for HPV and premalignant changes in the uterine cervix is highly recommended in the HIV-seropositive population [3].

Equally important is the fact that if high risk HPV types play a major role in the development of intraepithelial neoplasia, progression to the stage of infiltrating cancer depends also on secondary genetic alterations that can occur during the intraepithelial stage under the influence of diverse cofactors.

## INVASIVE CARCINOMAS

### HISTOLOGICAL AND VIRO-CLINICAL ASPECTS

Squamous cell carcinoma (> 70% of cervical cancers) and adenocarcinomas (25% of cervical cancers) are the two main cellular types of cervical uterine carcinomas. Other histological forms (small cell carcinomas, glassy cell carcinomas, villo-glandular adenocarcinomas) are rare. All these forms are commonly associated with specific sequences of HPV. In an extensive French series, the rate of association between HPV and cervical cancer was 96%, with types 16 and 18 representing 55.5% and 14.2% of the cases respectively [4]. A comparison between clinical and virological data demonstrated three core findings:

- there is a link between histological type and type of HPV;

HPV = human papillomavirus

HIV = human immunodeficiency virus

- about half the adenocarcinomas are associated with HPV-18
- the age of the patients, on average, differs according to the HPV genotype associated with the tumor: 45.8 years for women presenting a cancer associated with HPV-18, 48.3 years for women with HPV-16-associated tumor, and 53.6 years for women presenting a tumor associated with any other type of virus. In this last group, the prognosis of the disease is more often favorable, based on evidence of a slow evolutionary process and better response to the treatment
- approximately 4% of cervical cancers lack a detectable HPV sequence. These cases generally develop in older patients (average age 62.5 years), a fact to be taken into account in any screening policy.

### INFILTRATING CARCINOMAS: RECENT BIOLOGICAL DATA

Techniques of large-scale analysis of the transcriptome and the genome allow specification of the biological characters of cervical cancer.

#### ANALYSIS OF THE TRANSCRIPTOME

The E6/E7 oncoproteins, 150 and 100 amino acids respectively, are produced and secreted by HPV. These two oncoproteins inactivate two different tumor suppressor genes: *p53* (chromosome 17) is inactivated by E6; and *RB1*, retinoblastoma tumor suppressor gene, (chromosome 13) is inactivated by E7 [5]. The viral oncoproteins E6 and E7 are thought to modify the cell cycle in order to retain the differentiating host keratinocyte in a state that is favorable to the amplification of viral genome replication and consequent late gene expression [6].

The transcriptome analysis of certain types of cancer, such as breast cancer, defines molecular groups that correspond to different biological entities. Transcriptome analysis of cervical cancer did not show such heterogeneity; the molecular subgroups observed were found to overlap with histological types [7]. In particular, distinct signatures of tumors associated with HPV-16 or 18 have not been observed, suggesting that these two types of viruses exert their oncogenic potential according to very similar mechanisms. Regarding the prognosis, the analysis brought to light a group of genes implied in the regulation of cell proliferation. Interestingly, the level of expression of these genes was found to be positively correlated to that of the E7 HPV oncogene [7]. This information translates into the infiltrating cancer stage at the clinical level and deregulation of the cellular proliferation due to the role played by the viral gene. The E7 viral oncoprotein, necessary for the maintenance of the transformed phenotype, thus represents a potential target in cervical cancer.

### Circulating tumor DNA (ctDNA) in cervical cancer patients carries the same genetic and epigenetic information as the primary tumor

#### GENOME ANALYSIS

The techniques of comparative genomic hybridization shed light on the structural alteration of the tumor genome. The high level of resolution of comparative genomic hybridization allowed mapping of the various types of genomic abnormalities observed in cervical cancer. The genomic analysis showed that the profile changes differ according to histological type [8]. In contrast, no difference was found with respect to the viral genotype associated with the tumor. Contrary to what is observed in other types of neoplasia, genomic amplifications are infrequent in cervical cancers and rarely recur. Finally, these techniques are able to show the frequent occurrence of genomic alterations of carcinoma cells at the insertion site of HPV DNA sequences.

#### GENOME STRUCTURE ANALYSIS AT THE INSERTION SITE OF HPV DNA SEQUENCES

The change in the physical state of the HPV genome over time represents an important step in cervical oncogenesis. In intraepithelial neoplasia, the viral chromosome in the shape of a circular DNA molecule is released inside the nucleus of the infected cell in the form of multiple copies. In infiltrating cancers, this chromosome is generally inserted into the tumor cell chromosome, while the free forms are rare or absent.

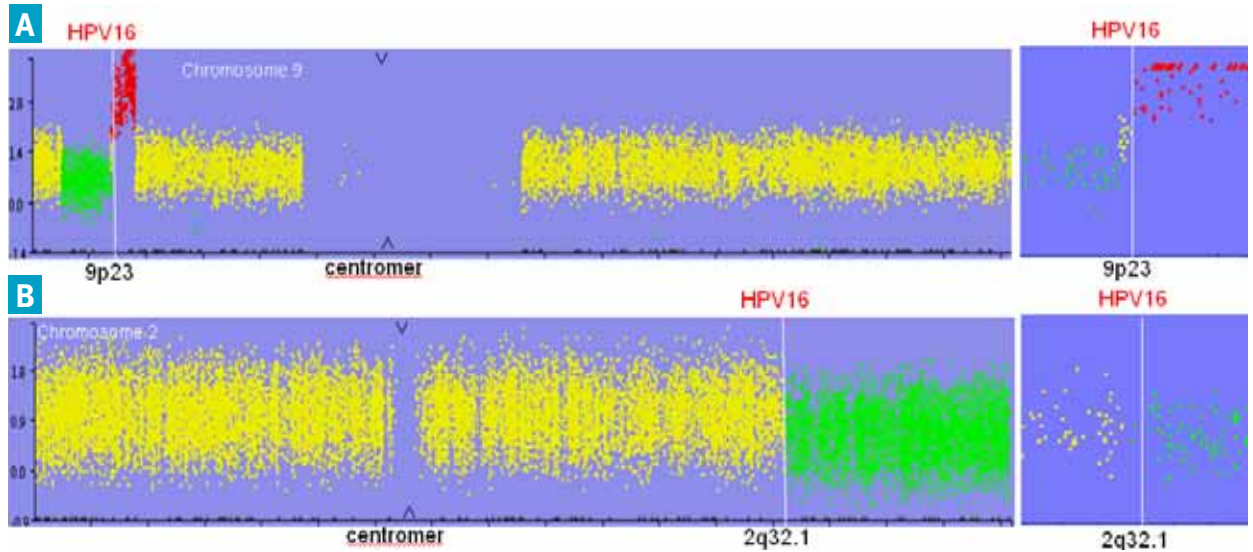
A large German study of the chromosome localization of the viral genomes performed over the last 10 years [9] showed that there was a marked plurality of the integration sites comprising all the chromosomal bands. However, for a given tumor, there is generally only a single site of integration, sometimes two, and unusually three.

This site of integration is clonal, meaning that it is present in all the tumor cells and is unchanged over time. In spite of the plurality of the observed sites of integration, certain recurring sites were identified and some examples of alterations of cellular genes localized close to inserted viral sequences were reported [10-12]. More recently, interesting data were obtained from a comparison of the results of analyses by comparative genomic hybridization and the localization of the sites of integration of the HPV genome [8]. This combined approach has allowed a detailed mapping of the cellular genome at the site of insertion of the viral sequences. Genomic rearrangements at the insertion site were observed in about 40% of the cases. These rearrangements were most often a local amplification [Figure 1A]. In other cases, the site of insertion coincides either with a loss of genetic material, segmental or distal [Figure 1B], or with a gain. It is almost certain that such rearrangements play an important role in tumor development and are secondary to the integration.

Experimental data show that activation of the viral origin of replication, always preserved in integrated sequences, plays

**Figure 1.** Data of comparative genomic hybridization (CGH) and localization of the site of insertion of the HPV-16 genome in two cases of cervical cancer. After CGH analysis, the normal contents in DNA are represented in yellow, content in excess in red (moved upward) and losses in green (moved downward). A vertical white bar indicates localization of the viral sequences. The additional unit to the right shows a detailed view of the contents in DNA in the site of viral insertion

**[A]** Genomic amplification at the site of insertion of the viral sequences on the short arm of chromosome 9 (band 9p23). The viral sequences are localized exactly at the junction between normal DNA content (some visible yellow points in the additional unit to the right) and an amplified content  
**[B]** Loss of genetic material downstream to the site of viral insertion in the long arm of chromosome 2 (band 2q31.2). Insertion of the viral DNA is associated with the loss of the telomeric extremity of the chromosome involved



a determinant role in the development of these rearrangements. This allows better understanding of the mechanism of genomic changes in the site of integration of the HPV. The results of these studies, beyond their contribution to our understanding of the bases of oncogenesis, highlight an important fact: the integration of viral DNA sequence into the genome represents an insertion mutation that is specific for every tumor cell and therefore for every patient. Due to the clonal character of this mutation, which is present in all tumor cells, as well as its stability over time, it is a tumoral marker that is particularly useful in clinics.

#### CIRCULATING TUMORAL NUCLEIC ACIDS: NEW MARKERS IN CANCER FOLLOW-UP

The presence of nucleic acids in the circulating blood is a long-known phenomenon [13], but its clinical application is more recent. It has been shown that DNA molecules derived from tumor cells are released into the neoplastic micro-environment, probably secondary to necrosis or apoptosis before reaching the bloodstream. This circulating tumor DNA presents the same genetic and epigenetic characteristics as the tumor of origin. Furthermore, the concentration of ctDNA

ctDNA = circulating tumor DNA

#### The concentration of ctDNA is proportional to the tumoral mass, which makes it a potential serum tumoral marker

is proportional to the tumoral mass [14], which makes it a potential serum tumoral marker. Any clinical application based on this principle requires specific molecular alterations secondary to the tumor. Due to its viral association, cervical cancer fits properly into this approach. Some pilot studies

have shown that sequences of viral DNA [15] or viral RNA [16] could be detected in the serum of advanced cervical cancer patients. However, due

to the lack of sensitivity and specificity of the methodology used, this research had a low clinical impact. A new type of approach based on the detection of the viral mutational insertion should provide an assay of high specificity. The genetic approach requires isolation of the viral insertion loci and development of a specific protocol for each patient. Regarding sensitivity, new methodological approaches are available [17,18].

#### THERAPEUTIC PERSPECTIVES

##### THE VIRAL EPITOPES: A SPECIFIC THERAPEUTIC TARGET

In principle, therapeutic vaccination is very different from prophylactic immunization. Prophylactic vaccine aims to activate the B cell immune system against viral capsid proteins that are generally lost at the stage of infiltrating cancer.

On the other hand, therapeutic vaccination aims at generating T lymphocytic clones specifically against epitopes of oncoproteins E6 and E7, always expressed in tumor cells. This approach has proved its efficacy in preclinical models. More recently, a trial carried out in patients presenting lesions of vulvar intraepithelial neoplasia demonstrated the capacity of the immune system to reject an established neoplastic lesion, limited to the in situ stage [1].

Is it possible to widen the indications of this treatment to invasive cancers and, if so, in what clinical situations? The recurrence of cervical cancer constitutes a clinical setting necessitating progress in therapeutic modalities. However, an immunotherapy protocol could be efficient only in patients with a limited tumoral mass. This reinforces the importance of an early relapse diagnosis, ideally in a preclinical phase. As seen above, the tools necessary for this approach are available.

#### PREREQUISITES FOR A CLINICAL PROTOCOL

What are the main obstacles to devising a clinical protocol with a reasonable chance of success? The first step is to precisely measure, in clinical practice, the level of sensitivity and specificity of circulating nucleic acid detection tests. These tests will have to be performed at the time of the diagnosis of cervical cancer and then repeated at regular intervals during the follow-up. The results must be compared with imaging data according to a well-standardized protocol. Such an evaluation will be based on the development of a large prospective multicenter study, comparing information on clinical examinations, imaging and biological analysis. The simultaneous evaluation of different biological parameters (circulating viral DNA, circulating viral RNA, circulating tumor DNA) and the prospective recording of the clinical data collected during the follow-up should allow us to measure the value of these new markers in practice and to define a strategy of the best analysis.

We shall attempt to estimate the prognostic value of the serum marker at the time of diagnosis, at the end of the treatment, and particularly during the follow-up, regarding the diagnosis of clinical relapse. The dynamics of the marker during the initial treatment should also be indicative of its predictive value, and of the quality of response to the chosen treatment. Regarding the vaccination protocol, the use of long peptides covering the entire sequences of the two viral oncoproteins E6 and E7 has demonstrated its potential in pre-invasive lesions. No scientific evidence has yet proven the treatment to be effective against invasive cancers, particularly relapsing cancers previously treated with chemotherapy and radiation. Various modalities must be explored to increase

the efficiency of this approach. The type of adjuvant therapy used and the administration of concomitant chemotherapy are suitable parameters for increasing the efficiency of this innovative approach.

#### CONCLUSION

The knowledge regarding HPV and cervical neoplasias acquired during the last 20 years has allowed substantial progress in the domains of screening and prevention. With regard to treatment, new perspectives are possible today. However, complementary bio-clinical research is still necessary, particularly for estimating the prognostic and predictive value of the new emerging serum markers derived from the molecular characterization of tumors.

Moreover, in the next few years, the development of high-throughput genotyping techniques will enable an optimal definition of these molecular alterations. The clinical value of this new information will be based, however, on the ability of the medical and technical teams to use it in a rational way. These teams must be able to guarantee the correct management and evaluation of the already available markers. An important amount of organizational work remains to be done; the ideal grounding will only be established through the right handling and analysis of sequential biological samples for rapid assimilation.

#### Acknowledgments

We thank Dr. Talia NG for her help preparing this manuscript

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