## Commentary

## Human papillomavirus (HPV) insertional mutation as a dynamic & specific tumour biomarker in HPV-associated carcinoma

The work reported by Das *et al*<sup>1</sup> in this issue is focused on the analysis of the physical status of human papillomavirus (HPV) genomes in cervical carcinoma, in comparison with the course of the disease. It is necessary to recall that the HPV genome, a 8-Kb circular molecule, may exist in two different physical forms according to the type of lesions. In benign papillomas as in most pre-invasive neoplasia, HPV DNA molecules are present as free episomes in the nucleus of keratinocytes whereas in most invasive carcinoma, viral DNA is integrated into the tumour cells genome<sup>2</sup>. Free molecules, heterogeneously distributed in tumour cells, may also be present in variable amount according to different cases. Integration disrupts the circular viral chromosome which is generally partially deleted. However, the viral E6 and E7 oncogenes are constantly conserved and expressed in tumour cells<sup>3</sup>. Das *et al*<sup>1</sup> have shown that in cervical cancers with integrated forms of HPV DNA, the disease outcome is worse than in cases harbouring only free molecules. How to explain this result and why is it an important one?

The analysis of different cellular models has shown that the integration of HPV DNA frequently interrupts the viral E2 gene which has negative regulation properties on expression level of the E6 and E7 viral oncogenes. Integration, *per se*, leads to the constitutive expression of the viral oncogenes and can be an important step in tumour progression. In addition, insertion of HPV DNA may also lead to a deregulation of cellular genes located at the vicinity of the integration locus. This deregulation may be secondary to structural changes such as focal amplification<sup>4</sup> or losses<sup>5</sup> of genes implied in tumour progression, or to the influence of the viral regulatory region on cellular genes located in the vicinity of the viral genome. Finally, it cannot be excluded that in certain cases, integration is a mere evidence of instability of the tumour genome and represents a biological parameter of the severity of the disease.

In the perspective of the current progress in the molecular characterization of solid tumours, three important points can be derived from the analysis of HPV genome status in tumours:

*(i)* HPV DNA is a tumour marker that should facilitate the detection of circulating tumour DNA (ctDNA) and improve the biological follow up of patients. Circulating viral DNA is an indirect tumour marker but the use of mutational insertion for the detection of ctDNA provides a more specific tumour marker<sup>6</sup>. Using HPV and cellular flanking sequences as a molecular marker, the detection of ctDNA will thus allow a highly sensitive and specific diagnosis of infraclinical relapses, and provides a tool for an optimal biological "personalized monitoring of patients".

*(ii)* HPV is not only a tumour marker but also a therapeutic target. This has been shown in immunotherapy protocols<sup>7</sup>. Moreover, recent data on *in vitro* genetic engineering show that the specific destruction of HPV oncogenes leads to a reversion of the tumour state<sup>8</sup>. These innovative treatments will be more efficient in cases with minimal tumour mass. And thus, the early detection of tumour relapse will be of paramount importance in the perspective of specific therapy, and the specificity of the alteration detected will be of crucial importance.

*(iii)* The advances on cervical cancer patients monitoring and treatment will be easily extended to other HPV-associated disease. In particular, HPV has been found to be associated with 88 per cent of carcinoma of the anal canal<sup>9</sup> and 40 per cent of head and of neck<sup>10</sup>. This latter tumour type will be particularly important since it is the world's seventh most common

type of cancer<sup>11</sup>, and HPV status is of strong prognostic significance<sup>12</sup>.

On the whole, the findings of Das *et al*<sup>1</sup> are important as these imply that teams of researchers and clinicians are together involved in the development of tumour markers. These teams will be able to include the leads obtained in their practice and in new therapeutic approaches of HPV-associated cancer in the very next future.

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