HPV HAS LEFT THE BUILDING – THE ABSENCE OF DETECTABLE HPV DNA AND THE PRESENCE OF R ALLELE/S FOR THE P72R POLYMORPHISM IN THE *TP53* GENE MAY CALL FOR MORE AGGRESSIVE THERAPEUTIC APPROACH IN HPV-ASSOCIATED TUMOURS

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ABSTRACT

HPV infection is a major pathogenetic factor in cervical carcinoma as well as in many of the squamous cancers of head and neck and other epithelial cancers. Persistence of HPV DNA detectable by routine methods is considered to be a risk factor for advanced CIN and, in patients treated by surgery or non-surgical treatment modalities (radiotherapy, chemotherapy), HPV persistence is believed to be associated with increased risk for local recurrence. In terms of survival, however, it has been repeatedly proven that patients with cervical cancer and other HPV-associated cancers with detectable HPV DNA tend to have better outcomes than patients with HPV-negative tumours. The P72R polymorphism in the human TP53 gene has been contemplated as an independent phenotype modifier in cancers, especially the R allele which has been shown to confer higher pro-apoptotic properties to the resultant p53 protein. It has been demonstrated, however, that RR homozygotes were much more common in study groups with HPV-associated tumours than the other two genotypes and that the P allele in P/R heterozygotes was preferentially lost while the R allele was preferentially retained and mutated. It is possible that HPV-dependent carcinogenesis strictly relies on the presence of HPV and the expression of the E6 and E7 oncoproteins only in the initial phases of transformation of infected cells (e.g. CIN). It may be associated with activation of latent HPV that would create a background of decreased control over the integrity of the genome of the host cell. The process can develop further by mechanisms independent of the presence of HPV and if the virus clears at some later point, that would not halt the already ongoing neoplastic transformation. Absence of HPV DNA in cervical tumours, whether before or after treatment, is not a reason to decrease vigilant monitoring and rule out the need for further treatment, as it may be quite possible that the TP53 gene of the infected cells has already been modified in the course of cancer progression by HPV-independent mutagenesis. Cervical tumours that are HPV-negative ought to alert attending oncologists for the possibility for increased growth potential and invasiveness of the tumour so as to contemplate more aggressive anticancer therapies, especially in carriers of the R allele of the P53R polymorphism.

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Significance of the presence of HPV in the dysplastic epithelium as a predictor of outcomes in HPV-related dysplasia and carcinoma

A plague upon it when thieves cannot be true one to another!
William Shakespeare, King Henry IV - Part I, act 2, scene 2

The origins of the carcinoma of the uterine cervix as well as of many of the squamous cell carcinomas of head and neck (SCCHN) and penile carcinomas can in most cases be traced back to an infection with human papillomavirus (HPV). The prevalence of HPV infection is very high, with about 80% of the adult population being infected with at some point in their lives with one or more types of HPV. Over 99% of the HPV infections are cleared in several months up to one year, with less than 1% of the cases with HPV infection persisting beyond 12 months eventually progressing to cervical intraepithelial neoplasia (CIN). A significant proportion of CINs of virtually any grade may clear on their own and only about one-third of the advanced dysplasies eventually progress to cervical cancer, which makes up for less than 0.1% of the initial HPV infections eventually resulting in carcinoma of the uterine cervix. Anticancer measures usually consist of watchful waiting often coupled with routine checks for HPV DNA in the cervical epithelium. Persistence of viral DNA beyond a fixed period of monitoring (usually 12-18 months) may prompt the patient and/or the attending gynaecologist to undertake more radical measures to eradicate the infection such as cervical conization. Persistence of HPV DNA in CIN lesions is considered to be associated with risk of progression to higher-grade CIN (4, 14). Nevertheless, persistence of viral DNA is not always correlated with the presence or the grade of dysplastic lesions typical of CIN or with cancer. In more than half of the high-grade CINs

and overt cervical cancers the presence of HPV DNA can be readily identified by routine methods such as the polymerase chain reaction (5, 27). The association between persistence of HPV infection and increased risk of development of epithelial dysplasia eventually resulting in cancer is easily conceivable, as the longer the viral genome is present in the infected cell, the higher the risk that it may reactivate and integrate into the host cell DNA, unleashing its transforming potential. Indeed, the chance for any rare event generally increases with time if the general dynamics of all other factors that may modify the chances for occurrence of the event is slow (as are potentially carcinogenic cellular changes related to aging) (10, 26). In theory, at least, this would mean that the longer HPV DNA persists, the higher are the risks for development of cancer. It is known, however, that HPV DNA may persist for a very long time without significant progression in CIN grade or transformation to overt cancer. Also, considering the theoretical model, the identifiable presence of HPV DNA in cervical cancers could be expected to be associated with worse prognosis than in tumours in which HPV DNA cannot be detected. Indeed, in patients with cervical cancer eligible either for surgical or for non-surgical treatment modalities such as radiotherapy it has been found the presence of HPV DNA after completion of treatment is associated with higher risk for local recurrence than in patients in which HPV DNA was undetectable after therapy (22, 32). It has been repeatedly demonstrated, however, that the prognosis in terms of survival after cervical cancers and head and neck cancers negative for HPV DNA may be actually worse than that of HPV-positive cancers (8, 24, 30). The presence of HPV DNA in the tumour tissue was actually proposed as a predictor for better outcome in some HPV-associated cancers, such as penile carcinoma (19). The paradoxical finding has been repeatedly confirmed and it was proposed that the aggressive nature of tumours in which HPV DNA was not detectable could be explained by somatic mutations (8). It was already known at the time that the carcinogenic properties of the E6 oncoprotein of HPV were related to its ability to inactivate one of the major signalling molecules responsible for the maintenance of the genome integrity and the activation of the apoptotic pathway in infected cells, p53 (36). What is more, cancer-specific variants of the *P53* gene producing p53 variant proteins which have acquired new functions compared to the wildtype p53 (gain-of-function mutations) have been found predominantly in HPV-related cancers in which HPV DNA was not detectable (8, 13, 23). Again, it could be expected that the presence of HPV in tumour cells ought to predict worse outcomes than its absence, as aggressive tumours often lack detectable p53 and the modification of the *TP53* gene is believed to constitute an important step in the progression between dysplasia and overt cancer. Still, the fact remains that patients with cervical cancer and other HPV-associated cancers with detectable HPV DNA tend to have better outcomes than patients with HPV-negative tumours.

The role of polymorphic variants of *TP53* gene in the succeptibility to cancer

The individual capacity for repair of DNA damage has begun to be considered an important factor in pathogenesis of cancer relatively recently, but nowadays it is believed that it plays a role as vital as any other factor and possibly even more significant than the role of well-studied potent environmental factors. As most cancers occur as a result of the interplay between individual genetic background, environmental impact and ageing-related loss of capacity to recognize and/or repair DNA lesions. However, it is now believed that carriership of genetic factor/s which are known to modify the risk for cancer development may not be enough to trigger or halt carcinogenesis on its own but may well increase or decrease the lifetime risk for cancer and/or modulate the outcomes of various therapies. Such genetic factors were readily identified among the polymorphisms in genes coding for products participating in the management of genome integrity and/or recognition of DNA damage, such as *p53, XPC, XPD, XPF, ERCC1* and others (3, 15, 17, 34). The P72R polymorphism in the human p53 gene is common in all populations, though the prevalence of the one or the other form may vary at different latitudes and in different racial groups, possibly with relation to the amount of ambient exposure to UV (3, 29). The two polymorphic variants have been pronounced to be conformationally indistinguishable and to possess practically the same sequence-specific DNA-binding capacity, i.e. to be both wildtype (35). The proline allele and the arginine allele, however, have been found to differ significantly (by a factor of 15) in their transactivation properties with regard to their target genes (11, 35). Specifically, it was stated that the R variant was a stronger inducer of programmed cell death in response to DNA damage while the P variant was a stronger inducer of transcription of downstream genes related to cell cycle arrest and attempt for repair of the damaged DNA and it was proposed that co-inheritance of the variant forms of *TP53* together with inherited cancer proneness phenotypes may modify the outcomes in individuals carrying different polymorphic variants of p53. The experimental proof of this theory came readily as it was demonstrated that the P72R PP homozygous carriers of heritable molecular defects predisposing to cancer phenotypes such as MSH2 or MLH1 mutations associated with hereditary nonpolyposis colon cancer (HNPCC) had earlier age of onset and a worse prognosis than the carriers of PR genotype and especially of the RR homozygous genotype (16). As the P72R polymorphism does not actually alter the capacity for recognition of damage by p53 but the possible outcomes of this recognition, it was assumed that increased capacity to induce apoptosis in cells whose DNA has been modified would work in clinically healthy individuals towards decreasing the general risk for cancer occurrence, regardless of the type of cancer. Since cancer is among the most common causes of mortality in the elderly, it could easily be inferred that lower propensity towards cancer would translate into an increase of longevity for

the carriers of the R allele. It has been shown, however, that in the group of the oldest old (>85 years) the incidence of cancer was actually increased almost twofold in PP homozygotes compared to the PR and RR carriers overall (28). At the same time the overall survival of PP carriers exhibited a significant increase (over 40%) in comparison to the R allele carriers (9). This was supposedly related to better post-cancer survival (25), possibly because of better response to treatment, as it was quite natural to assume that after cancer has already arisen, higher DNA repair capacity of the tumour cells may actually increase the risk for development of resistance to 'classic' antitumour therapies (which most often work by virtue of inflicting genotoxic damage). Then again, it could be expected that in the course of anticancer therapy the R allele of the P72R polymorphism (conferring better pro-apoptotic properties) would work to ensure more efficient removal of tumour cells damaged by genotoxic treatments. Recently, it has been shown that the P allele (PP and PR genotypes compared to the RR genotype) is associated with higher levels of accumulation of damage to mitochondrial DNA under conditions of induced oxidative stress and with higher levels of mitochondrial heteroplasmy (2). The former conforms to the theory that less pronounced pro-apoptotic potential may eventually result in accumulation of DNA damage (which may, in turn, trigger carcinogenesis), while the latter corroborates the statistics for P72R allele distribution in various age groups, in view of that mitochondrial DNA heteroplasmy is a commonly seen and heritable trait in the oldest old (>85) (38). Apparently, the expectations that lower capacity for DNA repair was always detrimental in terms of outcome were not met. What is more, it has been repeatedly demonstrated that the carriers of the R variant of the P72R polymorphism and especially the RR homozygotes were overrepresented (not underrepresented, as could be expected) in study groups with HPV-associated tumours (cervical carcinoma, SCCHN) compared with the normal population, regardless of the smoking status (the latter being regarded as a major environmental risk factor for HPV-related carcinogenesis) (7, 18, 21). The finding of higher percentage of RR homozygotes, however, was found to be correct for true carcinoma only and not for precancerous high-grade CIN (37).

The minus of a minus – why is that apparently deleterious factors may be associated with better prognosis

Cancers with HPV-related pathogenesis apparently exhibit paradoxical behaviours with regard to the presence of the causative factor. The usual rules seem not to apply for the association of DNA repair capacity and proneness to damage-induced apoptosis with cancer succeptibility as well. Worse outcomes have been found to be associated independently with absence of the DNA of the viral offender in tumours and with carriership of the seemingly possessed of stronger anticancer properties R form of p53. The two phenomena seems to have a common basis which lies, as could be expected in a condition with multifactorial pathogenesis such as cancer, in the relationship between factors of exogeneous origin (HPV oncoproteins) and factors of endogeneous origin (the stochastic character of mutability in cancer and the different properties of the variant wildtype forms of p53). In 1998, Storey et al. estimated the succeptibility to E6-related degradation of the R variant form of p53 to be several times higher than of the P variant (33). Later, Marin et al. demonstrated that mutant p53 carrying arginine in codon 72 was more likely to bind and inactivate normal p73 than the same mutant p53 but in its proline-containing variant (20). p73 is a p53-related protein which may bind to gene promoters usually bound by p53 and act as alternative activator of apoptosis in p53-deficient cells (12, 20). Marin et al. also reported that the Pro-containing p53 allele was preferentially lost while the Arg-containing allele was preferentially retained and mutated in squamous cell tumours arising in R/P heterozygotes (20). About half of all human cancers, including a significant proportion of HPV-related cancers, carry mutant variants of the *TP53* gene. Some of these are loss-of-function mutations (including deletions of the *TP53* locus or the 17p chromosome arm), allowing cells with damaged or altered DNA to progress through consecutive cell cycles instead of inducing cell cycle arrest and/or p53-dependent apoptosis in response to damage. Others are essentially gain-of-function, that is, the mutation does not result in inactivation of the wildtype p53 or physical removal of the locus containing the gene but in acquirement of new functions. The latter is typical of cancer variants of p53 and is believed to be one of the hallmarks of cancer (31). Unlike other cancers, in which loss of p53 function is a major step in cancerous transformation (e.g. colon cancer), in many HPV-associated tumours occurrence of gain-of-function mutations in p53 seems to represent the essential transition from epithelial dysplasia to carcinoma (13, 21). This has so far been faithfully reproduced in mouse models with SCCHN (1). The persistence of HPV DNA in precancerous states such as CIN may be associated with risk of progression to higher grades of dysplasia, as the longer the viral offender is present and active, the deeper the suppression of the normal cell mechanisms to prevent replication of altered DNA, and, respectively, the higher risk for further dysplastic changes in the affected epithelium. Similarly, in HPV-related tumours with preserved wildtype *TP53* gene the persistence of HPV after treatment may indeed point towards increased risk for cancer recurrence, as the E6/E6-associated protein complex of the HPV would presumably continue binding and tagging normal p53 for degradation. In cases, however, in which the *TP53* locus is lost (e.g. by deletion) or the transition between dysplasia and overt cancer is furnished by gain-of-function mutagenesis of *TP53*, the presence or absence of HPV in the tumour becomes less important, as one of the major targets of the viral oncoproteins is already taken down. This may be especially true for the carriers of the pro-apoptotic Arg variant of p53, as it was shown to be more mutation-prone than the

P variant (20, 21). Having the R allele of *TP53* selectively retained as a mutation target in cancer cells while the P allele is selectively lost might be an important micro-evolutionary decision of epithelial cells which have embarked on the way of neoplastic transformation, differentiating the high-grade dysplasia from carcinoma, as it was reported that the striking prevalence of RR homozygocity in tumours is valid for overt cervical cancer only and not for advanced CIN (37). This decision may be related, on the one hand, to the potentially detrimental function of the wildtype P-containing p53 in the progression of cancer; and on the other hand, on the higher propensity for mutagenesis of the R allele. This way, as the cancerous transformation is dependent on the number of cell cycles, the P allele which is involved in the major pathway of cell cycle arrest and damage repair is actively lost and the alternative pathways (e.g. the pRb-dependent mechanism which prevents damaged cells into entering S-phase) are inactivated via other mechanisms. At the same time, the *TP53* allele coding for p53 protein which is more readily inactivated by the E6 oncoproteins and which is more mutation-prone (the R allele) is retained. Therefore, the presence or absence of HPV would matter in terms of prognosis only in CIN (where the R-allele dependent mutagenesis producing gain-of-function p53 is supposedly not significant yet) and in the relatively small proportion of HPV-associated tumours in which the wildtype p53 sequence is preserved. It has been shown in SCCHN that HPV DNA-positive tumors rarely had TP53 gene mutations or general loss of chromosome arms and/or whole chromosomes, whereas the majority of tumours negative for HPV DNA had *TP53* mutations as well as large genomic deletions, including the 17p chromosome arm (6). It could be speculated that the presence of HPV and the expression of its major oncoproteins creates an overall background of genomic instability on which the collective function of the E6 and E7 proteins sustains the infected cell through enough cycles of replication of damaged DNA so as to grossly increase the chances for occurrence of a somatic mutation that may as well effectively eliminate or transform the function of p53. Beyond this point, the presence or absence of HPV DNA in the cell is immaterial, as the transformation process follows a different agenda though the ends are effectively the same. The presence of HPV DNA and the active transcription of the E6 oncoprotein in HPV-related tumours are therefore likely to be indicative of the presence of wildtype p53 eligible for inactivation, providing opportunities for anticancer intervention via p53-dependent apoptosis. The absence of HPV DNA in cervical tumours ought to alert attending oncologists for the possibility for increased growth potential and invasiveness of the tumour so as to contemplate more aggressive anticancer therapies, especially in carriers of the R allele of the P53R polymorphism.

Conclusions

HPV-dependent carcinogenesis is apparently strictly dependent on the presence of HPV and the expression of the E6 and E7 oncoproteins only in the initial phases of carcinogenic transformation, as the transformation process triggered by HPV genome integration can develop further by different mechanisms. The pro-carcinogenic action of the major oncoproteins E6 and E7 can only be important for the neoplastic transformation of the infected cells if and when the cellular proteins which are inactivated by these oncoproteins are coded by intact, wild-type cellular genes. In the majority of HPV-associated cancers the absence of HPV oncoproteins which would sustain a cancerous cell through multiple cell cycles has apparently been compensated for by somatic mutations, usually involving loss of genomic regions and gain-of-function mutations. This is especially valid for tumours carrying the mutation-prone R allele of the P72R polymorphism. Therefore, the presence of HPV DNA in HPV-associated cancers may serve as an indicator of additional opportunities for curative intervention. Screening for the P72R polymorphism in the *TP53* gene ought to be included in routine monitoring of patients with persistent HPV infection so as to provide additional information about possible outcomes.

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