Biomolecular and epidemiological aspects of human papillomavirus induced cervical carcinogenesis

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Chapter 7

General Discussion

Contents

Cervical carcinoma remains one of the leading causes of death from cancer among women worldwide[1]. Organised screening programmes aim to trace precursor lesions in order to reduce cervical cancer incidence. Human papillomavirus (HPV) is a necessary cause for cervical carcinogenesis. Most HPV infections are cleared and mild cervical abnormalities regress because of an efficient cellular immunity. A failing immunological surveillance eventually results in the development of cervical cancer.

This thesis describes and discusses the above mentioned aspects in cervical carcinogenesis. Several topics that are touched in the chapters are highlighted at this point in a general discussion.

1 Surinamese Environment and Cervical Cancer

Developing countries are high-risk areas for cervical cancer of which the incidence rates differ worldwide[1-5]. Suriname is such a high-risk area[6]. Both endogenous and exogenous factors can influence development of cervical (pre)malignancies and differences in incidence rates between geographical areas[7]. Important exogenous or environmental factors are HPV, screening history and sexual lifestyle[7-9]. HPV infection is more common in high-risk areas for cervical cancer[10-15], including Suriname[16]. A higher prevalence and viral load and different (more virulent) HPV type variants in a high-risk area might cause an increased risk of cervical cytological abnormalities, as suggested recently[17,18]. High HPV exposure could be associated with a sexual lifestyle encouraged by the Surinamese culture, as was established in several other populations[9,19-21]. Possible promiscuity of males or females clearly increases the risk of acquiring an HPV infection and it is suggested that the relatively young age at first intercourse could intensify the susceptibility to HPV persistence.

The high cervical carcinoma incidence in Suriname is reflected in the high prevalence of moderate and severe dysplasia which was observed in cervical smears from the first organised cervical screening programme in Suriname[Chapter 2]. The significance of environmental factors for differences in the geographical incidence of cervical cancer and its precursors was emphasised by a decreased prevalence of dysplasia in Surinamese immigrants in the Netherlands, a low-risk area for cervical cancer[Chapter 3]. This significance is also supported by studies comparing risk factors in areas with different cervical cancer incidences[18,22].

The majority of the decline in cervical carcinoma incidence rates in developed countries is attributed to the implementation of organised screening programmes[5,23,24]. The protective effect of previous screening operates independently from HPV[9,22]. An absent or an only recently started organised screening programme for cervical cancer could therefore explain a high prevalence of cervical lesions in an area, in this case Suriname.

In addition to the stated risk factors, several cofactors for progression of cervical HPV induced lesions have been reported. These include smoking, oral contraceptives, parity[7,8,20] and exposure to carcinogens in the household environment[9].

Along with the above mentioned environmental factors, endogenous aspects that mainly consist of immunological and genetic characteristics strongly influence the course of cervical carcinogenesis.

2 Significance of Multiple HPV Infections in Cervical Cancer

As said, premalignancies are mainly induced by HPV infection and persistence of oncogenic HPV is imperative for progression to cervical carcinoma. HPV persistence allows for expression of the HPV oncogenes E6 and E7, which is associated with the malignant progression of cervical neoplasia. The oncoproteins E6 and E7 deregulate cell cycle control mechanisms, create genomic instability, and can eventually cause aneuploidy[25,26].

In the multi-step cervical carcinogenesis with environmental, immunological and genetic factors, the role of multiple HPV infections is not immediately obvious. Until recently it was thought that multiple HPV infections were only present in premalignant cervical lesions, but now we know that invasive cervical tumours can also be infected by multiple HPV types. There appears to be no significant difference between high- and low-risk areas for cervical cancer, although the odds of having multiple HPV infections are higher in a high-risk population[Chapter 6].

The significance of multiple HPV infections in cervical carcinoma can be viewed in combination with viral integration and aneuploidy status. HPV integration is an important step in cervical carcinogenesis, but is not always necessary for the expression of E6 and E7[27]. It does, however, secure viral persistence, which is a prerequisite for expressing the viral oncogenes. Previously, HPV integration was investigated with PCR-based techniques, estimating integration by comparing the copy numbers of an often deleted (E1/E2) and a mostly preserved (E6/E7) viral oncogene. Recently several studies concerning HPV integration have been published that utilised fluorescence in situ hybridisation (FISH), which has the advantage of imaging episomal as well as integrated HPV. Most cervical carcinomas seem to have HPV present in the integrated form, occasionally accompanied by HPV episomes[28,29].

As stated above, the ultimate result of the deregulation of cell cycle mechanisms by the HPV oncoproteins is aneuploidy. It could be hypothesised that aneuploidisation occurs after viral persistence, making the tumour clone unstable and herewith facilitating HPV integration. This is suggested by Melsheimer *et al*. who showed that 19 out of 20 aneuploid lesions had integrated HPV[30]. However, one could also argue that viral integration increases genomic instability inducing aneuploidy. Evidence for both mechanisms was observed previously[31,32].

What role do multiple HPV infections have in cervical carcinogenesis? Apparently it is favourable for some cervical tumours to be infected with multiple HPV genotypes. An additional HPV type might result in sufficient oncogenicity even without viral integration in some tumours. In two out of three HPV 16/18 co-infected cervical carcinomas, we found viral integration of one type in the aneuploid fraction, but the third case showed presence of both types without HPV integration[Chapter 6].

To summarise, expression of the viral E6/E7 oncogenes does not require HPV integration [27], which is probably one of the main reasons that the timing of HPV integration in cervical carcinogenesis can vary. The presence of multiple, possibly synergistic, high risk-HPV genotypes could lead to extensive expression of the viral E6/E7 oncogenes. Aneuploidy weakens the genome and therefore enhances oncogenicity of a tumour in general as well as the integration of HPV. The main conclusion seems to be that there are multiple paths which can lead to progression of a cervical tumour, probably depending on individual immunogenetic and available environmental factors.

3 Immunogenetic Heterogeneity in Cervical Cancer

The cellular immune system is able to eliminate viruses and virus induced lesions. HPV induced cervical lesions are associated with a failing immunological surveillance which is performed by cytotoxic T-lymphocytes (CTLs), activated when human leukocyte antigen (HLA) class I antigen presents aberrant peptides. In addition, tumour progression appears to be facilitated by altered expression of cytokines among which are interferon and several interleukins, leading to decreased local cellular immunity[33-35].

Loss of HLA surface expression occurs frequently in cervical (pre)neoplasia[36-40]. The nature and frequency of HLA class I antigen loss mechanisms were elegantly studied in a group of freshly sorted cervical cancer samples and it was established that altered HLA class I expression was frequent, diverse, mainly caused by genetic changes and combined with widespread tumour heterogeneity[40]. The diversity and heterogeneity of HLA class I aberrations are illustrated by the expression patterns observed in cervical tumours and adjacent cervical intraepithelial neoplasia (CIN) lesions[Chapter 5]. Strong, weak and absent expression of HLA class I was observed in the samples, often varying within the cases. In most patients, CIN and invasive tumour samples provided similar results, supporting the hypothesis that both are from the same clonal process and demonstrating that HLA class I loss is an early event in cervical carcinogenesis. The failure to express HLA class I could result from loss of heterozygosity (LOH) at 6p21.3 in combination with a locus-restricted event in this area. Mutations in the HLA class I genes and in β_2m were described previously[40]. Larger deletions in the HLA class I area were not observed using FISH[Chapter 5]. It is likely that the HLA class I aberrations allow the premalignant CIN lesion to escape

immune surveillance and progress to invasive cancer.

Not all cases with loss of HLA class I expression can be explained by genetic defects in the HLA class I or β_2m genes. Low transporter associated with antigen processing (TAP) expression has previously been reported and was found to be associated with loss of HLA class I expression in cervical carcinomas[41-43], but until recently information about the underlying mechanisms was limited. Previously reported TAP mutations in the majority of cervical carcinoma samples emerged to be polymorphisms and LOH[44]. In our study altered TAP expression was observed in more than 40% of the cases, in two cases accompanied by a 1 bp deletion in the 5'-UTR[Chapter 6]. Only some of the adjacent precursor lesions displayed loss of TAP expression, indicating that the timing of TAP downregulation varies between cervical tumours. In several cases the loss of TAP expression was more extensive in CIN than in the invasive tumour tissue. This could implicate different clonal origins or the fact that only TAP negative CIN lesions survived T-cell attack activated by the tumour's presence. All samples with altered TAP expression displayed a heterogeneous staining pattern of scattered nests of TAP positive among TAP negative tumour cells, even in the cases with the 5'-untranslated region (UTR) somatic mutation and LOH. This type of pattern probably results from aberrations in regulation rather than from clonal expansion of TAP negative and positive tumour cell populations. Most TAP and associated HLA class I aberrations can be upregulated through interferon stimulation[45,46], although structural TAP alterations in tumour cells have been established recently[47-49]. TAP defects can diminish the HLA class I cell surface expression, but there is increasing evidence that an effective antiviral defence can occur via TAP independent mechanisms as well[50-54].

4 Conclusions

In the present thesis we focused on multiple aspects of cervical carcinogenesis in developed and developing countries. Several conclusions can be made based on the studies presented. The high cervical carcinoma incidence in Suriname is reflected in the high prevalence of moderate and severe dysplasia which was observed in a sample of cervical smears from the first organised cervical screening programme in Suriname. This can be attributed to the absence of an organised screening programme for cervical cancer until recently, which is associated with high prevalence of cervical lesions in an area. In addition, the decreased prevalence of dysplasia in first-generation Surinamese immigrants in the Netherlands illustrates the significance of environmental factors for differences in the geographical incidence of cervical cancer and its precursors.

Looking at endogenous aspects of cervical carcinogenesis it is important to realise that the timing of HPV integration in cervical carcinogenesis can vary, but the viral oncogenes always need to be expressed. The cellular immune system recognises HPV infected

lesions. Selective pressure results in HLA defective tumour cells and a failing immune surveillance increases cervical tumour progression. The frequent HLA class I aberrations occurring in cervical carcinoma are diverse and heterogeneous and partly associated with TAP alterations.

Hitherto, the largest decrease in cervical cancer incidence has been accomplished by organised cervical screening programmes. The success of organised screening programmes is conditional upon a high response rate and regular screening intervals, which unfortunately remains very difficult to realise in developing countries where cervical cancer is the leading cancer among women. In addition, the effect of successfully implemented screening programmes can first be observed after some time (decades). The large number of HPV vaccination studies illustrates the current focus in cervical cancer research. Prophylactic HPV vaccines show a protection of 70% against the high-grade cervical lesions. A prophylactic HPV vaccine seems a promising solution in the near future for developing countries in establishing a substantial decrease in cervical carcinoma incidence. However, the frequently aberrated immune system in women with cervical cancer suggests a difficulty in establishing an effective immunisation by therapeutic HPV vaccines, which should be taken into account in further research concerning these vaccines.

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