Alterations in AP-1 and AP-1 regulatory genes during HPV-induced carcinogenesis

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Abstract. Background: Previous studies demonstrated a functional involvement of the AP-1 transcription factor in HPV-induced cervical carcinogenesis. Here, we aimed to obtain further insight in expression alterations of AP-1 family members during HPV-mediated transformation and their relationship to potential regulatory (Notch1, Net) and target (CADM1) genes.

Methods: mRNA expression levels of c-Jun, JunB, junD, c-Fos, FosB, Fra-1, Fra-2, Notch1, Net and CADM1 were determined by quantitative RT-PCR in primary keratinocytes (n = 5), early (n = 4) and late (n = 4) passages of non-tumorigenic HPV-immortalized keratinocytes and in tumorigenic cervical cancer cell lines (n = 7). In a subset of cell lines protein expression and AP-1 complex composition was determined.

Results: Starting in immortal stages c-Fos, Fra-2 and JunB expression became up regulated towards tumorigenicity, whereas Fra-1, c-Jun, Notch1, Net and CADM1 became down regulated. The onset of deregulated expression varied amongst the AP-1 members and was not directly related to altered Notch1, Net or CADM1 expression. Nevertheless, a shift in AP-1 complex composition from Fra-1/c-Jun to c-Fos/c-Jun heterodimers was only observed in tumorigenic cells.

Conclusion: HPV-mediated transformation is associated with altered AP-1, Notch1, Net and CADM1 transcription. Whereas the onset of deregulated expression of various AP-1 family members became already manifest during the immortal state, a shift in AP-1 complex composition appeared a rather late event associated with tumorigenicity.

Keywords: Cervical cancer, HPV, AP-1, Fra-1, c-Fos, Net, Notch1, CADM1

1. Introduction

Cervical carcinogenesis is initiated by infection with high-risk human papillomavirus (hrHPV) types, in particular HPV types 16 and 18 [21,42]. However, infection with hrHPV alone is not sufficient for the progression to cervical cancer. In fact, in vitro studies have shown that HPV-induced carcinogenesis is characterized by consecutive stages of transformation reflected by altered phenotypes such as immortalization, anchorage independent growth and tumorigenicity, which necessitate (epi)genetic alterations within the host cell genome [8,33]. Somatic cell fusion studies have shown that progression through each of above mentioned stages is particularly dependent on recessive processes, indicating that deficiencies in tumor suppressor gene activity are key events [8]. As an example, fusion of tumorigenic cervical carcinoma cells with normal fibroblasts led to a reversion to a non-tumorigenic phenotype [32]. In order to define the chromosomes involved, microcell mediated chromosome transfer studies have been performed on HPV transformed cells representing various phenotypes [2,24,36]. It appeared that human chromosome 11 reduced cell growth in soft agar and/or tumorigenicity of cervical carcinoma cell lines SiHa and HeLa without affecting the immortal phenotype [15,27,35].

Allelotyping studies on cervical carcinomas have identified a high frequency of loss of heterozygosity (LOH) at two loci on chromosome 11, i.e. 11q13 [14,30] and 11q22–q23 [4,13,20,23]. In recent studies we found that the tumor suppressor gene Tumor Suppressor in Lung Cancer 1 (TSLC1), recently renamed
as CADM1 (cell adhesion molecule 1), which is localized at 11q23.2, is functionally involved in HPV-induced transformation. We showed that restoration of CADM1 expression in SiHa cells resulted in a suppression of anchorage independent growth and tumorigenicity [35]. However, given previous cell fusion experiments it is unlikely that CADM1 silencing alone is sufficient to drive the progression from an immortal to a tumorigenic phenotype. One other gene that might be involved in this process encodes a member of the Fos gene family, Fos Related Antigen 1 (Fra-1), which is located on chromosome 11q13. In addition to Fra-1, the Fos gene family consists of c-Fos, FosB, and Fra-2. These Fos family members encode proteins that form heterodimers with proteins encoded by the Jun family (c-Jun, JunB or JunD), resulting in a so-called AP-1 transcription factor [10]. This complex is involved in the positive and/or negative regulation of several different genes, including CADM1, depending on its composition [10].

Various studies have shown that changes in AP-1 complex composition are involved in transcriptional regulation of HPV during cervical carcinogenesis [7, 16, 17, 26]. Additionally, alterations in AP-1 complex composition have been associated with the tumorigenic phenotype of the cervical carcinoma cell line HeLa that displayed high c-Fos expression, while Fra-1 expression was almost undetectable [26]. Whereas in HeLa cells and tumorigenic segregants of HeLa-fibroblast hybrids (CGL3 cells) the AP-1 complex consisted of c-Fos/c-Jun heterodimers, non-tumorigenic HeLa-fibroblast hybrids (444 cells) displayed Fra-1/c-Jun heterodimers [28, 29]. A functional involvement of AP-1 in tumorigenicity of cervical cancer cells is supported by the finding that ectopic expression of c-Fos in 444 cells resulted in a tumorigenic phenotype, which was accompanied with changes in AP-1 composition [28]. An up regulation of c-Fos and down regulation of Fra-1 was also shown in other tumorigenic cervical cancer cell lines such as SiHa and SW756, as well as in cervical carcinomas [25, 29].

Recently, increased c-Fos expression in cervical carcinoma cell lines SiHa, HeLa and SW756 was shown to result from the loss of expression of the TCF transcription factor Net [41]. In addition, down regulation of Fra-1 in tumorigenic cervical carcinoma cells has been suggested to result from deregulated Notch1 expression [39, 40]. All the above described findings suggest that both CADM1 silencing and an altered composition of the AP-1 complex represent key events in the progression from an immortal to a tumorigenic phenotype, the latter representing the in vitro counterpart of tumor invasion in vivo. However, present data on alterations in AP-1 complex composition during HPV-induced carcinogenesis are mainly derived from primary human cells, a limited number of cervical carcinoma cell lines and somatic cell hybrids of carcinoma cells and fibroblasts. At present neither it is known at what stage and in which order during HPV-induced carcinogenesis these changes occur nor how they relate to CADM1 silencing.

We have previously transfected primary human keratinocytes with full-length HPV types 16 and 18, resulting in the establishment of four immortal keratinocyte cell lines, i.e. FK16A and FK16B containing HPV16, and FK18A and FK18B containing HPV18 [38]. These cell lines resemble high-grade cervical precursor lesions in organotypic cultures [37] and their genetic profiles closely overlap with those found in cervical (pre)malignant lesions [38, 45]. Consequently, these cell lines represent a good model system for cervical carcinogenesis, allowing a longitudinal analysis of alterations involved in HPV-mediated transformation. In this study we used this model to investigate when and in what order expression of AP-1 complex encoding and regulatory genes may change during transformation, and how these expression changes relate to CADM1 silencing.

2. Materials and methods

2.1. Cell lines and cell culture

Primary human keratinocytes were isolated from foreskins of 6 donors as described before [38]. The establishment of the HPV16 and HPV18 immortalized cell lines (FK16A, FK16B, FK18A and FK18B) has been described previously [38]. The cervical carcinoma cell lines SiHa, HeLa and CaSki, were obtained from the American Type Culture Collection (Manassas, VA, USA). HeLa hybrids 444 and CGL3 were kindly provided by Prof. Dr. E.J. Stanbridge (University of Irvine, Irvine, CA, USA). HeLa hybrids 444 and CGL3 were kindly provided by Prof. Dr. E.J. Stanbridge (University of Irvine, Irvine, CA, USA). The cervical carcinoma cell lines 778, 808 (both containing HPV18), 866, and 879 (both containing HPV16) were kindly provided by Prof. Dr. P.L. Stern (Paterson Institute for Cancer Research, Manchester, UK). All cells and cell lines were cultured as described previously [5, 35, 38, 41].
2.2. RNA isolation and real-time RT-PCR

RNA was isolated using either the RNeasy kit (Qiagen, Hilden, Germany) or RNA Bee (Tel-Test Inc., Friendswood, Texas, USA) according to the manufacturer’s manual. To exclude amplification of residual DNA, RNA isolates were treated with RQ1 DNase (Promega, Leiden, The Netherlands) according to the manufacturer’s directions. Real-time RT-PCR for CADM1 was performed on the LightCycler (Roche Diagnostics, Woerden, The Netherlands) as described previously [35]. To correct for differences in RNA quality and input, we performed RT-PCR for housekeeping gene porphobilinogen deaminase (PBGD) as described before [44].

For real-time RT-PCR of the remaining genes cDNA was prepared using an oligoDT primer (Invitrogen, Breda, The Netherlands) and AMV Reverse Transcriptase (Promega). Real-time PCR for c-Fos, Fra-1, Fra-2, c-Jun, JunB, Notch1, Net and the housekeeping gene snRNP was performed on the ABI/Prism 7700 Sequence Detector System (Taqman-PCR; Perkin Elmer/Applied Biosystems, Warrington, United Kingdom) and AMV Reverse Transcriptase (Promega). All primers and probes were selected using Primer Express 2.0 (Applied Biosystems, Warrington, United Kingdom) or taken from the RT-primer database of the University of Gent in Belgium (http://medgen.ugent.be/rtprimerdb). The following primers base of the United Kingdom or taken from the RT-primer database of the University of Gent in Belgium (http://medgen.ugent.be/rtprimerdb). The following primers

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\begin{align*}
\text{c-Fos} & : 5'\text{GCTCGACCTCAGGAGGATGAGTAT}3' \\
\text{Fra-1} & : 5'\text{ACCTCCAGACGTTCCTTCTCTT}3' \\
\text{JunB} & : 5'\text{TCGAGGCAAGACAGATCA}3' \\
\text{JunD} & : 5'\text{CTTGCTCAGCCAGACTA}3' \\
\text{Notch1} & : 5'\text{GCCCGGGTCCTCCTAGAAA}3' \\
\text{Net} & : 5'\text{CCACACTCACACGTGGGTA}3' \\
\text{snRNP} & : 5'\text{TCCAGGCGGAGACAGATCA}3' \\
\end{align*}
\]

and for Fra-1. For all targets potential amplification of

\[
\begin{align*}
\text{c-Fos} & : 5'\text{CTGGCTCAGGCAG}3' \\
\text{Fra-1} & : 5'\text{CCC-GAGGAGGGTTGGA}3' \\
\end{align*}
\]

was controlled for by both treatment of RNA isolates with RQ1 DNase and inclusion of cDNA reactions without the addition of reverse transcriptase. These latter control reactions were always negative.

All reaction mixtures contained 12.5 µl 2× Sybr Green master mix (Perkin Elmer/Applied Biosystems), primers at 0.5 µM and cDNA prepared from 30 ng RNA in a total volume of 25 µl. The following reaction conditions were used: 95°C for 10 minutes and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

In each amplification round a dilution series of RNA derived from primary keratinocytes ranging from 1250 ng to 125 pg was included to generate a standard curve. For each sample the Ct was determined, i.e. the cycle number at which the amount of amplified target crossed a fixed threshold. The amount of mRNA of a specific gene in each sample was intrapolated from the standard curve using the Ct value. The relative expression of a gene of interest was calculated by dividing the amount of mRNA of that specific gene by the amount of snRNP U1A mRNA. Relative expression levels in primary keratinocytes were set to 100% by the following formula: (GENE/snRNP) primary keratinocytes/(GENE/snRNP) HPV containing cells/(GENE/snRNP) primary keratinocytes × 100%. Each run contained two reactions per specimen per gene. Mean expression values were used for calculations. Additionally, we confirmed reproducibility by performing additional runs for each gene of interest on a subset of the specimens.

2.3. Nuclear extracts and western blot analysis

Nuclear extracts for Western blot analysis and EMSA were prepared as described previously [1]. Protein concentration was determined by the Bradford method using bovine serum albumin as a standard. Nuclear extracts were separated on a 10% SDS-polyacrylamide gel, and electrotransferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA, USA) and probed with the following antibodies: c-Fos (06-341, Upstate Biotechnology, Santa Cruz, CA, USA) and probed with the following antibodies: c-Fos (06-341, Upstate Biotechnology), Santa Cruz Biotechnology, Santa Cruz, CA, USA) and probed with the following antibodies: c-Fos (06-341, Upstate Biotechnology). Bands were visualized with horseradish peroxidase-conjugated anti-Rabbit IgG or anti-mouse IgG using the ECL+ detection system (PerkinElmer Life Sciences Inc., Wellesley, MA, USA).
2.4. Electrophoretic mobility shift assays (EMSAs)

AP-1 consensus oligonucleotide 5′-CGCTTGA-TGACTCA-GCCGGAA-3′ [19] was generated in an Applied Biosystems synthesizer using phosphoramidite chemistry and further purified by HPLC. The annealed oligonucleotide was labelled with 32P-γ-ATP (Amersham, 3000 Ci/mmol) with T4 polynucleotidokinase and purified from a 15% polyacrylamide gel. The binding reaction was performed as described before [9]. After 30 min incubation at room temperature, 2 µg of specific antibody were added and incubated at 4°C for 1 hour. Antibodies used were: c-Jun (sc-822X), Fra-1 (sc-183X) and c-Fos (sc-52X) all from Santa Cruz. The complexes were resolved on 5.5% nondenaturing polyacrylamide gels, by running 30 min at 280 V followed by 75 min at 350 V. Gels were dried and exposed to X-ray films.

3. Results

3.1. Altered expression of AP-1 complex genes during HPV-mediated carcinogenesis

To investigate how expression of AP-1 complex encoding genes changes during HPV-induced carcinogenesis, we analyzed their mRNA expression levels in five isolates of primary keratinocytes (EK), early and late passages of four HPV-immortalized keratinocyte cell lines (FK16A, FK16B, FK18A, FK18B), and seven cervical carcinoma cell lines. Whereas all HPV-immortalized cells were previously found to be non-tumorigenic, all carcinoma cell lines induce tumor formation in nude mice [35]. mRNA expression of the Fos family members c-Fos, Fra-1 and Fra-2, and the Jun family members c-Jun and JunB was analyzed by real time RT-PCR. Expression of JunD and FosB could not be analyzed, due to undetectable mRNA levels.

Compared with primary keratinocytes, c-Fos mRNA levels were increased in early passages of the HPV-immortalized cells and in cervical carcinoma cell lines, but not in late passages of the HPV-immortalized cells (Fig. 1(a)). Fra-2 mRNA levels gradually increased from primary cells to the tumorigenic cells (Fig. 1(b)), whereas Fra-1 mRNA expression was found to gradually decrease during transformation (Fig. 1(c)). Also for c-Jun, a gradual down regulation of mRNA expression was observed, which was though less pronounced and became apparent from the late passage HPV-immortalized cells onwards (Fig. 1(d)). For JunB, an increase in mRNA expression is seen in both HPV-immortalized cell lines and in cervical carcinoma cell lines (Fig. 1(e)).

3.2. Changes in Net, Notch1 and CADM1 mRNA expression during HPV-mediated transformation

An up regulation of c-Fos mRNA expression in cervical carcinoma cells has been shown to result from reduced expression of Net [41]. Moreover, down regulation of Fra-1 has been suggested to result from deregulated Notch1 expression [40]. Since both c-Fos and Fra-1 expression changed substantially with progression towards tumorigenicity, we next investigated Net and Notch1 mRNA levels in the same RNA isolates of all cell lines.

As depicted in Fig. 1(f) Net mRNA levels were yet reduced in late passages of HPV-immortalized cells, and this reduction became even more prominent in cervical carcinoma cell lines.

For Notch1, a different expression pattern was observed. In HPV-immortalized cell lines, especially at late passages, an increase of Notch1 mRNA levels was found. In cervical carcinoma cell lines, however, Notch1 mRNA levels decreased again, not only compared to HPV immortalized cell lines but also compared to primary keratinocytes (Fig. 1(g)).

For CADM1 we previously reported a strongly reduced mRNA expression in cervical carcinoma cell lines compared to early passages of HPV-immortalized cells and primary keratinocytes [35]. To relate CADM1 mRNA levels to expression of AP-1 family members, we quantified CADM1 mRNA levels as well. Figure 1(h) shows that CADM1 mRNA levels decreased in late passages of the HPV-immortalized cell lines and became virtually undetectable in the cervical carcinoma cell lines.

3.3. Changes in AP-1 composition are associated with tumorigenicity and not immortality

To determine how altered mRNA expression levels correlated to protein levels, Western blot analysis for c-Fos and Fra-1 was performed on a subset of cell lines. Non-tumorigenic HeLa-fibroblast hybrids (444), and tumorigenic segregants of these 444 cells (CGL3), were included as a control [31]. As shown in Fig. 2A c-Fos was expressed in all cells analyzed, with increased expression seen in cervical carcinoma cell line SiHa, whereas Fra-1 protein expression was evident in both primary keratinocytes and HPV-immortalized cell lines but not in SiHa cells. These data are in line with the RT-PCR results indicating that mRNA levels and protein levels correlate well. Additionally, we detected Fra-1 expression but not c-Fos expression in 444
cells and c-Fos expression but not Fra-1 expression in CGL3 cells, which is in agreement with previous findings [29].

Previous studies have demonstrated a direct correlation between changes in AP-1 composition and tumorigenicity of HeLa cells. In tumorigenic HeLa cells showing hardly any Fra-1 protein expression, the AP-1 complex consisted of c-Fos/c-Jun heterodimers. In 444 cells on the other hand Fra-1 protein expression was increased and the AP-1 complex was found to contain Fra-1/c-Jun heterodimers. CGL3 cells displayed reduced Fra-1 protein expression and a reversal of the AP-1 complex into c-Fos/c-Jun heterodimers [28,29].

To examine whether the observed changes in c-Fos and Fra-1 expression in our model reflected an altered composition of the AP-1 complex we performed Electrophoretic Mobility Shift Assays (EMSAs) in combination with antibodies specific for c-Jun, Fra-1 and c-Fos on primary keratinocytes, late passages of HPV immortalized cell lines, SiHa, 444, and CGL3 cells (Fig. 2B). In both primary keratinocytes and HPV immortalized cell lines (upper panels), a ‘supershift’ was
seen upon addition of c-Jun and Fra-1 specific antibodies, indicating that the AP-1 complexes mainly exist as Fra-1/c-Jun heterodimers. Notably, although c-Fos was expressed to a certain extent as detected by Western blot in these cells (Fig. 2A), no incorporation of c-Fos in the AP-1 complex could be detected by EMSA.

In SiHa cells on the other hand application of c-Fos and c-Jun resulted in a ‘supershift’ pointing to an AP-1 complex consisting of c-Fos/c-Jun heterodimers (lower left panel on the left) as was previously also shown for HeLa cells [29]. In the lower middle and right panels it is shown that in 444 cells a ‘supershift’ was observed upon addition of c-Jun and Fra-1 specific antibodies and in CGL3 cells upon addition of c-Jun and c-Fos specific antibodies. These data indicate that the AP-1 complex consisted of Fra-1/c-Jun heterodimers in 444 cells and c-Fos/c-Jun heterodimers in CGL3 cells, as was also found previously [29].

4. Discussion

Previous reports have demonstrated that both alterations in the composition of AP-1 transcription factor and CADM1 gene silencing are associated with the tumorigenic phenotype of HPV-transformed cells [28, 29,35]. However, it was unknown at what stage during HPV-induced malignant transformation alterations in AP-1 occur and how they relate to potential AP-1 regulatory genes, such as Net and Notch1.
Fig. 2. Primary keratinocytes (EK), late passages of the HPV-immortalized cell lines FK16A, FK16B, FK18A, FK18B, and SiHa, 444, and CGL3 cells were harvested at confluency. (A) Western blot of nuclear extracts (15 µg/lane) using 10% SDS-PAGE gels and antibodies against Fra-1, c-Fos and actin as a loading control. Molecular masses are indicated in kDa. (B) EMSAs using 32P-labeled AP-1 consensus oligonucleotides and 2 µg of c-Jun, Fra-1 or c-Fos antibody for the ‘supershifts’. The AP-1 and retarded complexes are marked by arrows.
In the present study we have shown that mRNA expression levels of the AP-1 family members c-Fos, Fra-1, Fra-2, c-Jun and JunB change at different stages during malignant transformation of primary keratinocytes by hrHPV. mRNA expression of c-Fos, Fra-2 and JunB increased during HPV-mediated transformation, whereas Fra-1 and c-Jun expression levels dropped with progression towards malignancy. The onset of the altered expression levels varied among the different genes. Whereas Fra-1 and JunB changed early on following immortalization, alterations in Fra-2 and c-Jun expression occurred in late immortal cells.

Interestingly, a biphasic change in c-Fos expression was seen, that ultimately resulted in high c-Fos levels in cervical carcinoma cells. The cause of this phenomenon is still unclear and awaits further elucidation.

Most prominent changes in the expression levels of the AP-1 complex encoding genes were observed in the tumorigenic cells and were reflected by an altered composition of the AP-1 complex. Whereas in primary cells and HPV-immortalized cells c-Jun/Fra-1 heterodimers were found, in tumorigenic cells heterodimers consist primarily of a c-Jun/c-Fos composition.

Our findings support the data from previous studies describing an inverse correlation between Fra-1 and c-Fos expression in cervical carcinoma cells resulting in an AP-1 complex consisting of c-Jun/Fra-1 heterodimers in non-tumorigenic hybrid cells and a shift to c-Jun/c-Fos heterodimers in tumorigenic segregants [29,41]. Interestingly we did observe elevated c-Fos levels in a subset of the non-tumorigenic HPV-immortalized cells, indicating that the presence of c-Fos does not necessarily result in the formation of c-Jun/c-Fos heterodimers, as has also been demonstrated before by Rösl et al. [26]. Decreased Fra-1 mRNA levels on the other hand were exclusively found in the tumorigenic cells, indicating that composition of the AP-1 complex is more likely to be dependent on Fra-1 expression levels, rather than on c-Fos expression levels. Using RNA interference mediated knockdown of Fra-1 expression in the immortal cells it would be interesting to determine whether changes in Fra-1 expression are indeed sufficient for a shift in AP-1 complex composition from Fra-1/c-Jun to c-Fos/c-Jun heterodimers and an induction of tumorigenicity.

We showed that an inverse correlation between c-Fos and Net expression was restricted to tumorigenic cells, as demonstrated previously in tumorigenic versus non-tumorigenic HeLa-hybrids [41]. The present finding that a down regulation of Net was yet evident in late immortal cells, can be explained by the fact that Net can act both as a repressor and as an activator of c-Fos transcription depending on its phosphorylation status. Phosphorylated Net activates c-Fos transcription and unphosphorylated Net represses c-Fos transcription [6]. Further studies will reveal whether the phosphorylation status of Net differs between late passage immortal cells and carcinoma cells.

mRNA levels of Notch1, a potential regulator of Fra-1 expression [40], were found to only partially correlate with Fra-1 mRNA levels, suggesting other regulators to be involved as well. Moreover, the finding that a peak in Notch1 mRNA expression was seen in late passages of HPV-immortalized cells, which dropped again in cervical carcinoma cell lines, may in part explain the discrepancies between studies describing Notch1 having transforming capacity [18,43] and acting as a tumor suppressor gene [39,40]. The report by Weijzen et al. [43] showing oncogenic activities of Notch1 concerned CaSki cells. In the present study the AP-1 and Notch1 expression levels in CaSki were found to be opposite to all other cervical carcinoma cell lines analyzed, i.e. elevated Fra-1, reduced c-Fos and elevated Notch1 mRNA levels. In a second study involving other carcinoma cell lines Notch1 over expression inhibited proliferation, suggestive of Notch1 having tumor suppressive activities in late stages of transformation [18]. This latter finding matches with the observed down regulation of Notch1 in all carcinoma cell lines, except from CaSki in the present study. On the other hand, the up regulation of Notch1 in the HPV immortalized cell lines suggests that Notch1 may be involved in early stages of cellular transformation, as was also demonstrated by Lathion et al., showing that low expression of Notch1 in primary keratinocytes induced transformation [18].

Finally, we aimed to relate changes in CADM1 mRNA levels to changes in AP-1 dimer composition. Similar to the alterations in AP-1 family members and regulators, particularly c-Jun, Fra-2 and Net and to a lesser extent Fra-1, CADM1 down regulation became apparent in late passage immortal cells and was further decreased in the cervical carcinoma cell lines. A possible interconnection is supported by a recent publication in which CADM1 has been identified as a target of the AP-1 transcriptional complex [11]. Our recent studies have shown that promoter methylation also contributes to CADM1 silencing in cervical cancer cells [35]. It will be of interest to determine whether and how an altered AP-1 complex composition affects CADM1 transcription in HPV-transformed cells.
and how this is related to promoter methylation at the different stages of transformation. Since CADM1 has not only been shown to suppress tumorigenicity but also anchorage independent growth this also raises the question whether an altered AP-1 complex composition affects anchorage independent growth as well.

With respect to the latter it is of note that the viral protein E2 has recently been demonstrated to regulate MMP-9 expression, a well known regulator of tumor invasion, via an induction of AP-1 activity [3]. Moreover, next to E2 also HPV16 E6 has been demonstrated to modulate AP-1 activity [22]. Consequently, it will be interesting to examine whether the shift in AP-1 composition as observed in tumorigenic cells is related to altered expression levels of the viral proteins.

Up until now AP-1 complex proteins have primarily been studied in cervical carcinoma cell lines and their derivates. The present study has provided more insight in the status of these genes in immortal, not yet tumorigenic cells, which have previously been demonstrated to closely resemble premalignant cervical lesions [34]. We showed that for a subset of AP-1 members as well as for Net and Notch1 the onset of deregulated expression AP-1 expression occurred in these immortal cells. These cells therefore provide a valuable tool for further functional studies to elucidate the mechanism underlying the altered expression regulation of AP-1 during HPV-mediated transformation.

In conclusion, we demonstrated that HPV-mediated transformation is associated with altered AP-1 transcription. Whereas the onset of deregulated expression varied amongst the AP-1 members, a shift in AP-1 complex composition was restricted to tumorigenic cells.

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