Biochemical and Biophysical Research Communications 412 (2011) 20–25

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Differential expression of cellular microRNAs in HPV 11, -16, and -45 transfected cells

Anita Dreher [a,1], Maria Rossing [b,1], Bogumil Kaczkowski [c,1], Ditte K. Andersen [a], Therese Juhlin Larsen [a], Mikael Kronborg Christophersen [a], Finn Cilius Nielsen [b], Bodil Norrild [a,*]

[a] Institute of Cellular and Molecular Medicine, DNA Tumor Virus Laboratory, University of Copenhagen, Panum Institute, Blegdamsvej 3, 2200 Copenhagen, Denmark
[b] Department of Clinical Biochemistry, Copenhagen University Hospital, Blegdamsvej 5, 2100 Copenhagen, Denmark
[c] The Bioinformatics Centre, Department of Biology and Biomedical Research and Innovation Centre, Copenhagen University, Ole Maaloes Vej 5, 2200 Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 11 June 2011
Available online 18 July 2011

Keywords:
miRNA
HPV 11
HPV 16
HPV 45
Microarray

ABSTRACT

Human papillomaviruses (HPVs) are highly prevalent giving rise to both benign and malignant lesions why they are classified as high- and low-risk viruses. In this study we selected one low-risk (HPV 11) and two high-risk (HPV 16 and -45) types for genomewide miRNA analysis to investigate possible common and distinct features in the expression profiles. For this purpose we developed a cell culture model system in HaCaT cells for expression of the viral genomes under standardized conditions. We identified 25 miRNAs which were differentially regulated in two or three HPV types where 13 miRNAs were in common for all three types. Among the miRNAs identified, miR-125a-5p, miR-129-3p, miR-363, and miR-145 are related to human cancers. Noteworthy, miR-145 is found upregulated in the miRNA profiles of both high-risk HPV types. For selected differentially expressed miRNAs in HPV 16 predicted miRNA target transcript involved in signal transduction, RNA splicing and tumor invasive growth were validated by qRT-PCR. In addition, our results imply that the early 3′ untranslated region (3′UTR) of the three HPV genomes were not a target for miRNA regulation.

1. Introduction

The last decades have shown that human papillomaviruses (HPVs) are involved in several malignant and benign diseases. They cause highly proliferative infections and are dependent on the differentiation of their host cells where the virus infects the basal cell layer in cutaneous and mucosal epithelium. Dependent on their degree of oncogenecity they are divided into high-risk and low-risk types [6,16]. The high-risk types, such as HPV 16, -18, -45, and -33 belong to the genus α-papillomavirus species 7 and 9 and infections with these viruses can cause malignant transformation of the host cell. HPV 16 and -18 are the most frequently detected HPV types in anogenital cancers, especially in the cervix [20] and in head and neck cancers [8,10]. The low-risk types, such as HPV 6 and -11 cause benign condylomas and papillomas which can be highly distressing for the infected individuals [4]. The oncogenecity of the high-risk types is dependent on the constitutive expression of the oncogenes E6 and E7 and their following inactivation of p53 and pRB respectively [15,32]. Due to low binding affinity, E6 and E7 do not induce degradation of p53 and pRB, in low-risk HPV types [22]. HPV dependent malignancy is believed to depend on integration of the viral genome into the host cell chromosomes. This leads to disruption of the viral E2 gene, encoding a transcription factor able to regulate the expression of the viral oncoproteins E6 and E7. The upstream regulatory region (URR) has four binding sites for E2 and the transcription factor can up-regulate or inhibit transcription of the early HPV mRNAs [21,29,30].

MicroRNAs (miRNAs) interact with important cellular processes such as signal transduction, apoptosis and cell cycle progression. MiRNAs influence target mRNAs by binding to the 3′UTR and hereby regulating gene expression [3]. The regulatory effects of miRNAs are likely to be involved in both regulation of viral growth and cancer progression [33]. Several human cancers, e.g. breast cancer (for review see [12]), colorectal cancer [1], and head and neck cancer [9,23], show changes in the expression profiles of miRNAs. *In vitro* studies have shown certain miRNAs to be differentially expressed in HPV infected cells and cell lines [33]. Interestingly, several studies have demonstrated differential expression of miRNAs in invasive HPV positive squamous cell carcinomas and HPV positive cell lines compared to control tissue [13,14].

We have recently developed a model system in HaCaT cells in order to analyze the HPV mediated changes in cellular pathways and miRNA expression profiles in optimized growth conditions [7]. In the present study we examined the differentially expressed miRNAs in three selected HPV types; the low-risk HPV 11 and the

* Corresponding author. Address: Panum Institute, Blegdamsvej 3C, Building 22.3, 2200 Copenhagen, Denmark.
E-mail address: bnorrild@sund.ku.dk (B. Norrild).
[1] These authors contributed equally to this publication.

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.07.011

high-risk HPV 16 and -45. We found common characteristics in the miRNA expression profiles as well as distinctly expressed miRNAs among the three examined HPV types. Moreover, specific HPV regulated miRNAs were analyzed for regulatory function on target transcripts. Finally, a functional luciferase experiment was carried out to explore possible effects of selected miRNAs on the 3′UTR of the viruses.

2. Materials and methods

2.1. Cell culture and transfections

The human keratinocyte cell line HaCaT was chosen for the present study. The cells were grown at 37 °C and 5% CO_2. For cell cultivation Dulbecco's Modified Eagle Medium (DMEM 1965, Invitrogen) supplemented with 10% Fetal Calf Serum, 1% Penicillin/Streptomycin, 1% Glutamate and 1% 0.1 M sodium pyruvate was used. Transfections were done using jetPEI™ transfection reagent (Polyplus transfections). For stable transfection the circularized genome (6 μg) was transfected together with the pSV2-neo selection vector (2 μg) into HaCaT cells seeded 2×10^6 cell per 10 cm plate. As control, cells were transfected with the selection vector alone. The cells were grown under standard conditions using the same passage number and maintained under selection with G418 sulfate (Invitrogen) in a concentration of 500 μg per ml.

2.2. RNA and microarray analysis

The transfected cells grown under selection for 3 weeks were harvested for total RNA using the TriZOL reagent (Invitrogen) according to the manufacturer's protocol. RNA was analyzed by the Affymetrix and Exiqon_V.11.0 miRNA platforms. Details regarding labeling, hybridization, scanning procedures, and data pre-processing were carried out as described previously [7]. A heatmap was generated based on analysis of variance (ANOVA), for variance filtering ($q = 0.2$).

2.3. Statistical analysis

Differentially expressed miRNAs between each HPV type versus control were identified by means of empirical Bayes moderated *t*-statistics as implemented in LIMMA [26]. *p*-Values were adjusted for multiple testing by Benjamini and Hochberg False Discovery Rate method and *p*-values <0.05 were considered significant.

2.4. Cloning HPV and control 3′UTRs into the luciferase vector

The pCDNA3.1puro-dsLuc2cp *Firefly* and corresponding pCDNA3.1puro-hRluc-cp *Renilla* vector were kindly supplied by Christopher S. Sullivan, University of Texas at Austin. The 3′UTRs of HPV 11 (nt. 4370–4640), HPV 16 (nt. 4100–4310) and HPV 45 (nt. 3875–4323) were PCR-amplified from plasmids given as a kind gift from Prof. Harald zur Hausen, DKFZ, Heidelberg. The primer sequences used for PCR amplification were as follows (restrictions sites for *XhoI* and *XbaI* are underlined).

HPV 11 FW, 5′-GCATGGAC<u>CTCGAG</u>GAGTAAACCTTTTTTATACAG-3′ and RV, 5′-GCATGGAC<u>TCTAGA</u>CTTCCCAAGGGTATATACC-3′;

HPV 16 FW, 5′-GCATGGAC<u>CTCGAG</u>TGTATATGTACATAATG-3′ and RV, 5′-GCATGGAC<u>TCTAGA</u>CCTGCCTGTTTGCATGTTTTAT-3′;

HPV 45 FW, 5′-GCATGGAC<u>CTCGAG</u>AATCTGTATATTGTATAC-3′ and RV, 5′-GCATGGAC<u>TCTAGA</u>CAGGGGGGCACGTACC-3′ (Eurofins MWG Operon).

After cloning all constructs were sequenced (Eurofins MWG Operon).

2.5. Target gene analysis using qRT-PCR

Primers for the genes IKKα, IKKβ, ASF/SF2 and MTSS1 were designed and validated. Primers used were as follows.

IKKα FW, 5′-CGAAAGCTGCTCAACAAAC-3′ and RV, 5′-CCACCAACATCCTGAAGAAC-3′;

IKKβ FW, 5′-AAACCAGCATCCAGATTGAC-3′ and RV, 5′-AGCCATCATCCGTTCTACC-3′;

ASF/SF2 FW, 5′-TCCAGACATGCGAACCAAGGAC-3′ and RV, 5′-TCGAACTCAACGAAGGCGAAG-3′;

MTSS1 FW, 5′-GAAATAACCCACCTTCAGACC-3′ and RV, 5′-CCTTTCAAGTCCAGAATCACC-3′.

qRT-PCR was done with 90 ng of RNA extracted from HPV 16 transfected HaCaT cells selected for three weeks. qRT-PCR was done using the QuantiTect SYBR Green RT-PCR Kit (Qiagen). Relative quantification of the miRNA expression was calculated with the $2^{-\Delta\Delta C(T)}$ method [25].

2.6. Target analysis using Luciferase assay

HaCaT cells were seeded in 24-well plates at 1×10^5 cells per well 24 h prior transfection. For transfection 1 ng of pCDNA3.1-puro-dsLuc2cp with or without HPV 3′UTR insert, 1 ng pCDNA3.1-puro-hRluc-cp vector and 1 μg pBLUESCRIPT II SK (+) vector were mixed and co-transfected with 30 nM specific pre-miRNA (Ambion) or negative control#1 (scrambled miR) (Ambion) using jetPEI™ transfection reagent following the manufacturer's protocol (Polyplus Transfection). Luciferase activity was measured 24 h post transfection using the Dual-Luciferase® Reporter Assay System (Promega).

3. Results

3.1. Comparison of differentially expressed miRNAs

HaCaT cells were transfected with circularized HPV 11,-16, or -45 genomes as previously described [7]. Same batch and passage of transfected HaCaT cells were used for all studies. RNA obtained from each HPV type was analyzed on Affymetrix and Exiqon microarray platforms. Only miRNAs expressed with a fold change (FC) >1.5 and $p < 0.05$ were considered significant. For comparative studies we mainly focused on results based on the Affymetrix platform as this provided the most consistent expression values. The recently published HPV 11 miRNA expression profile (Affymetrix platform) is used for comparison of profiles between HPV 16 and -45 [7]. Our microarray experiment resulted in 50, 22, and 47 differentially expressed miRNAs in HPV 11, -16, and -45, respectively. Among these, only 13 miRNAs are shared between all three HPV types. Of the shared miRNAs, miR-181a, -125a-5p, -502-3p, -923, -92a-1*, and -500* are upregulated and miR-558, -576-3p, -606, -886-3p, -888, -1255a, and 1274b are downregulated. HPV 16 and -45 have three upregulated miRNAs in common; miR-145, -29b-1*, and -1246. HPV 16 and -11 share three downregulated miRNAs; miR-454*, -363, and -129*. Lastly, HPV 11 and -45 share five upregulated miRNAs; miR-455-3p, -331-3p, 15b, -1231, and -1180 and one; miR-129-3p is downregulated. Results of shared differentially expressed miRNAs are depicted in Fig. 1 and Table 1. The complete lists of differentially expressed miRNAs, including results from the Exiqon microarray platform are listed in Supplementary Tables S1A and S1B. The numbers of miRNAs identified by both platforms show some similarities. However, different array systems are based on different probe design and intensity of features will vary between platforms. The ANOVA analysis ($q = 0.2$) show 65 miRNAs representing the largest variance among the three HPV types and the HPV negative controls, as illustrated in

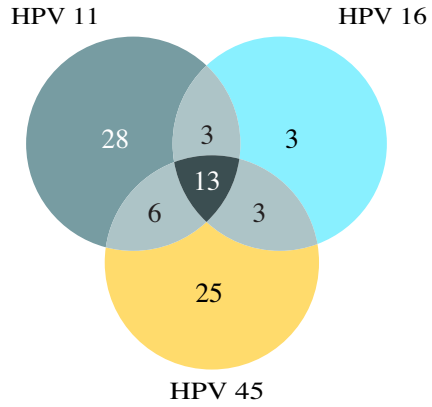

Fig. 1. Venn diagram showing the number of significantly changed miRNAs ($p < 0.05$) in HPV 11, -16 and -45. For the complete list of differentially expressed miRNAs see Supplementary Table S1A. The overlap between the individual types is seen and annotated miRNAs are listed in Table 1.

Fig. 2. It clearly shows several miRNA clusters unique for the various examined HPV-types. In particular it is noticeable that there are more clusters in common between HPV 16 and -45, reflecting the oncogenic potential of these two high-risk viruses opposed to the benign HPV 11.

3.2. Biological function of differentially expressed miRNA

As HPV 16 is the most prevalent type in premalignant and malignant diseases we searched for biological targets among differentially expressed miRNAs. By using computationally predicted mRNA targets in the TargetScan database (www.targetscan.org). Genes involved mainly in signal transduction, RNA splicing, post-transcriptional modifications and tumor metastasis. The chosen miRNA target genes, the miRNAs and their respective Fold Change are listed in Table 2. The expected inverse expression of the miRNA target genes was confirmed by qRT-PCR for all transcripts (IKKα, IKKβ, ASF/SF2 and MTSS1).

To study the possible effect of differentially expressed miRNAs on the 3′UTR of the selected HPV genomes, bioinformatic analysis of potential miRNA seed sequences (6-mer target sites) in HPV 11, -16 and -45 was done by Prof. Anders Lund's Research group. This analysis resulted in identification of a high number of potential binding sites for miRNAs. Only binding sites matching the differentially expressed miRNAs obtained by the array analysis were considered for further studies. After insertion of the 3′UTR sequences from each HPV type downstream of the luciferase reporter gene (U-LUC) we analyzed the putative miRNA regulation on HPV gene expression. Interestingly, none of the examined miRNAs showed any regulatory function. The results obtained for HPV 11 which was potentially targeted by miR-331-3p, -637, -874, -1274b, and -1275 are shown in Supplementary Fig. S1 (similar experiments were done for HPV 16 and -45, data not shown).

4. Discussion

Several studies have focused at the analysis of miRNA profiles in cultured HPV positive and negative cells as well as in clinical specimens from cervical cancer [24,33,34], nasopharyngeal cancer and oral cancer [2,9]. One study has reported that HPV modulates miRNA in a differentiation dependent process in order to maintain the viral replication capacity [14]. The mechanisms behind HPV mediated deregulation of the host cells are not completely understood and we therefore developed a reference cell culture model system.

The miRNA profiling of HPV 11, -16 and -45 transfected cells identified differentially expressed miRNAs. Interestingly, there were just 13 miRNAs regulated in common by all three types. However, among the most upregulated miRNAs, miR-125a-5p is known to be regulated in several cancers such as head and neck and gastric cancer. In gastric cancer a high level of the miRNA is related to a better prognosis for survival and can therefore be used as a prognostic factor [18].

Among the commonly downregulated miRNAs miR-886-3p is intriguing as this non-coding RNA molecule has recently been reclassified into a vault RNA even though it is still debated whether

Table 1
Significantly changed miRNAs shared between two or three HPV-types. Listed fold changes and expression levels derive from the Affymetrix platform.

	miRNA ID	Fold change			Average expression
		HPV11	HPV16	HPV45	
HPV16/45/11	hsa-miR-181a	1.8	1.7	2.1	8.5
	hsa-miR-125a-5p	3.3	2.6	3.8	8.4
	hsa-miR-502-3p	1.7	1.8	2	6.6
	hsa-miR-923	2.2	2.3	2.7	9.2
	hsa-miR-92a-1*	1.7	2.1	2.8	5.4
	hsa-miR-500*	2	2	2.2	6.5
	hsa-miR-558	−1.7	−1.9	−1.6	3.9
	hsa-miR-576-3p	−3	−3.4	−3.2	5.4
	hsa-miR-606	−1.9	−2	−1.7	4.9
	hsa-miR-886-3p	−10.3	−3.4	−4.4	7.2
	hsa-miR-888	−1.9	−2.3	−1.8	4
	hsa-miR-1255a	−1.9	−2.1	−2.2	4.3
	hsa-miR-1274b	−2.3	−2	−2.1	5.7
HPV16/45	hsa-miR-145		2.2	2.3	7.1
	hsa-miR-29b-1*		1.8	1.8	6
	hsa-miR-1246		2	2.7	8
HPV16/11	hsa-miR-454*	−2.9	−2.5		
	hsa-miR-363	−1.7	−1.6		
	hsa-miR-129*	−1.8	−1.9		
HPV11/45	hsa-miR-455-3p	2.1		1.7	7.5
	hsa-miR-331-3p	1.6		1.6	5.3
	hsa-miR-15b	2.1		2.6	9.8
	hsa-miR-1231	3.8		1.6	5.8
	hsa-miR-1180	1.8		2.1	5.7
	hsa-miR-129-3p	−1.7		−2	4.9

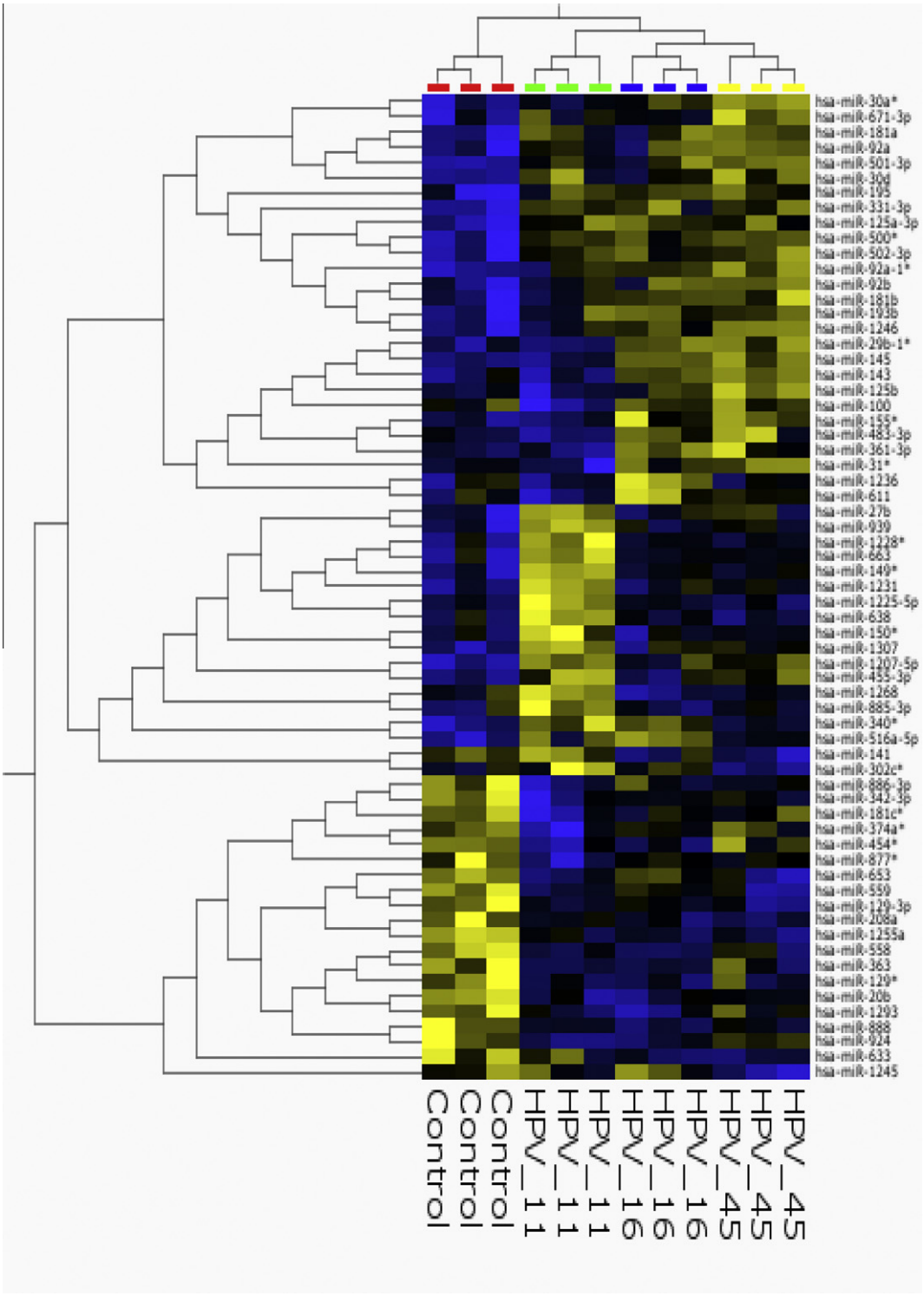

Fig. 2. Heatmap showing the result of the ANOVA analysis ($q = 0.2$). The 65 miRNAs correspond to the miRNAs with the largest variance between HPV 11, -16, -45, and control. Relatively low expression levels are represented as blue and relatively high expression levels are represented in yellow.

it is a true vault RNA as it behaves differently from other vault RNAs [11]. This non-coding RNA molecule is also regulated by other viruses such as Herpes viruses. Epstein-Barr-Virus infected cells increases, whereas Cytomegalovirus decreases the expression level of the molecule ("miR-886-3p") [17]. Moreover, suppressed expression of "miR-886-3p" is often seen in human cancer [11]. It will be interesting to learn more about the biological function of vault RNAs which could unveil why the three HPV types down-regulate the "miR-886-3p" up to 10-fold.

In the high-risk HPV 16 and -45 a twofold upregulation was shown for miR-145. Previous studies on HPV 16 positive cell lines showed no significant expression of miR-145 but when examined in raft cultures this miRNA was upregulated and therefore miR-145 may well correlate to cell differentiation [33]. In a recent study

Table 2
qRT-PCR validation of predicted miRNA target transcripts for four selected genes. Fold changes (FC) for miRNAs originate from the Exiqon microarray platform results.

Target mRNA	miRNA ID	FC miRNA	FC target mRNA
IKKα	miR-23a	1.5	−1.7
IKKβ	miR-16	1.4	−1.3
	miR-198	1.7	
ASF/SF2	miR-27b	−1.6	1.4
	miR-542-3p	−1.7	
MTSS1	miR-23a	1.5	−1.5

on head and neck cancers miR-145 was downregulated which might reflect dedifferentiation of tissue [10]. In line with these observations, previous studies revealed miR-145 as a common oncomiR in human cancers [1].

Among the downregulated miRNAs shared between HPV 11 and -16, miR-363 is downregulated 1.7-fold whereas studies in HPV positive pharyngeal squamous cancers show minor upregulation of miR-363 [10]. These opposing results could reflect the differences between cell culture models and *ex-vivo* cancer specimens. As a final point, miR-129-3p is differentially expressed in both HPV 11 and -45 and a recent study show miR-129–3p to be silenced by methylation as the DNA sequences contains CpG-islands upstream of the miR-locus in gastric cancer tissue [31].

To understand the biological function of changes in miRNA profiles, it is important to identify relevant target genes involved in regulation of either the virus life cycle or malignant progression of the host cell. In this study we selected genes involved in signal transduction, RNA splicing and tumor invasive growth. The gene ASF/SF2 has previously been shown to be of importance for expression of the late HPV 16 mRNAs [27] which is in line with the observed inverse correlation to the levels of miRs-27b and -542-3p in our model. The MTSS1 gene encodes a metastasis suppressor, and it is interesting that this gene is downregulated by miR-23a in the present study. Although it has not been discussed in HPV carcinogenesis it correlates to previous observations in bladder cancer [19]. Target analysis of miR-23a, -16 and -198 predicted the two IKK subunits to be target genes and our analysis confirmed the inverse mRNA expression of IKKα and IKKβ. The IKK complex is known to be modified by protein binding of HPV16 E7 oncoprotein [17,28]. The E7 protein binding to the IKK subunits reduces the NF-κB activity which is involved in cell proliferation [28]. It will be of interest to study the interplay of specific miRNAs and E7 for the regulation of the IKK subunits. The miRNA might confer a default regulatory pathway for fine tuning of the IKK level. Moreover, decreased NF-κB activity leads to tumor progression in skin cells, shown in a mouse model [5] and this finding could relate to our present HaCaT cell model as these cells originate from human skin. In summary, the present miRNA profiling provides a basis for further analysis of unknown biological pathways regulated by HPV. In this way new knowledge on common and individual features of the high- and low-risk HPV types may be discovered by analyzing the biological function of the distinct miRNAs found in this study. Finally, we found no indications that the 3'UTR of examined HPVs are targeted by miRNAs.

Acknowledgments

The technician Melissa Visser was supported by a grant from Laege Sophus Carl Emil Friis og hustru Olga Friis' Legat. Her technical assistance and that of Lucia Gavnholdt is acknowledged. The work was funded by, Lykfeldts Legat, Brødrene Hartmanns Fond, Snedkermester Sophus Jacobsen og hustru Astrid Jacobsens Fond, and Gangstedfonden, Dagmar Marshalls Fond og Fabrikant Einar Willumsens Fond. The Novo Nordisk foundation (Bogumil Kaczkowski) and student grants from the Danish Cancer Society supported Anita Dreher, Ditte K. Andersen and Mikael Kronborg Christophersen. Professor Anders Lund, Ulf A. Ørum, Lea Gregersen, BRIC, University of Copenhagen, Dr. Christopher S. Sullivan, University of Texas at Austin, and Professor Harald zur Hausen, DKFZ, Heidelberg are acknowledged for their help, useful discussions as well as for reporter constructs.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.07.011.

References

[1] Y. Akao, Y. Nakagawa, T. Naoe, MicroRNAs 143 and 145 are possible common onco-microRNAs in human cancers, Oncol. Rep. 16 (2006) 845–850.
[2] M. Avissar, B.C. Christensen, K.T. Kelsey, C.J. Marsit, MicroRNA expression ratio is predictive of head and neck squamous cell carcinoma, Clin. Cancer Res. 15 (2009) 2850–2855.
[3] R.W. Carthew, E.J. Sontheimer, Origins and Mechanisms of miRNAs and siRNAs, Cell 136 (2009) 642–655.
[4] L.T. Chow, T.R. Broker, B.M. Steinberg, The natural history of human papillomavirus infections of the mucosal epithelia, APMIS 118 (2010) 422–449.
[5] M. Dajee, M. Lazarov, P. Khavari, NF-kappa B blockade and oncogenic Ras induce invasive human epidermal neoplasia by circumventing cell cycle arrest, J. Invest. Dermatol. 119 (2002) 220.
[6] E.M. de Villiers, C. Fauquet, T.R. Broker, H.U. Bernard, H. zur Hausen, Classification of papillomaviruses, Virology 324 (2004) 17–27.
[7] A. Dreher, M. Rossing, B. Kaczkowski, F.C. Nielsen, B. Norrild, Differential expression of cellular microRNAs in HPV-11 transfected cells. An analysis by three different array platforms and qRT-PCR, Biochem. Biophys. Res. Commun. 403 (2010) 357–362.
[8] M.J. Lace, J.R. Anson, A.J. Klingelhutz, J.H. Lee, A.D. Bossler, T.H. Haugen, L.P. Turek, Human papillomavirus (HPV) type 18 induces extended growth in primary human cervical tonsillar or foreskin keratinocytes more effectively than other high-risk mucosal HPVs, J. Virol. 83 (2009) 11784–11794.
[9] C.B. Lajer, F.C. Nielsen, L. Friis-Hansen, B. Norrild, R. Borup, E. Garnaes, M. Rossing, L. Specht, M.H. Therkildsen, B. Nauntofte, S. Dabelsteen, B.C. von, Different miRNA signatures of oral and pharyngeal squamous cell carcinomas: a prospective translational study, Br. J. Cancer 104 (2011) 830–840.
[10] C.B. Lajer, B.C. von, The role of human papillomavirus in head and neck cancer, APMIS 118 (2010) 510–519.
[11] K. Lee, N. Kunkeaw, S.H. Jeon, I. Lee, B.H. Johnson, G.Y. Kang, J.Y. Bang, H.S. Park, C. Leelayuwat, Y.S. Lee, Precursor miR-886, a novel noncoding RNA repressed in cancer associates with PKR and modulates its activity, RNA 17 (2011) 1076–1089.
[12] N. Lynam-Lennon, S.G. Maher, J.V. Reynolds, The roles of microRNA in cancer and apoptosis, Biol. Rev. Camb. Philos. Soc. 84 (2009) 55–71.
[13] I. Martinez, A.S. Gardiner, K.F. Board, F.A. Monzon, R.P. Edwards, S.A. Khan, Human papillomavirus type 16 reduces the expression of microRNA-218 in cervical carcinoma cells, Oncogene 27 (2008) 2575–2582.
[14] M. Melar-New, L.A. Laimins, Human papillomaviruses modulate expression of microRNA 203 upon epithelial differentiation to control levels of p63 proteins, J. Virol. 84 (2010) 5212–5221.
[15] K. Munger, W.C. Phelps, V. Bubb, P.M. Howley, R. Schlegel, The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes, J. Virol. 63 (1989) 4417–4421.
[16] N. Munoz, f.x. Bosch, S.S. de, R. Herrero, X. Castellsague, K.V. Shah, P.J. Snijders, C.J. Meijer, Epidemiologic classification of human papillomavirus types associated with cervical cancer, N. Engl. J. Med. 348 (2003) 518–527.
[17] C. Nandy, J. Mrazek, H. Stoiber, F.A. Grasser, A. Huttenhofer, N. Polacek, Epstein-barr virus-induced expression of a novel human vault RNA, J. Mol. Biol. 388 (2009) 776–784.
[18] N. Nishida, K. Mimori, M. Fabbri, T. Yokobori, T. Sudo, F. Tanaka, K. Shibata, H. Ishii, Y. Doki, M. Mori, MicroRNA-125a-5p is an independent prognostic factor in gastric cancer and inhibits the proliferation of human gastric cancer cells in combination with Trastuzumab, Clin. Cancer Res. 17 (2011) 2725–2733.
[19] S. Nixdorf, M.O. Grimm, R. Loberg, A. Marreiros, P.J. Russell, K.J. Pienta, P. Jackson, Expression and regulation of MIM (missing in metastasis), a novel putative metastasis suppressor gene, and MIM-B, in bladder cancer cell lines, Cancer Lett. 215 (2004) 209–220.
[20] W.C. Phelps, J.A. Barnes, D.C. Lobe, Molecular targets for human papillomaviruses: prospects for antiviral therapy, Antivir. Chem. Chemother. 9 (1998) 359–377.
[21] W.C. Phelps, P.M. Howley, Transcriptional transactivation by the human papillomavirus type-16 E2 gene-product, J. Virol. 61 (1987) 1630–1638.

[22] D. Pim, L. Banks, Interaction of viral oncoproteins with cellular target molecules: infection with high-risk vs low-risk human papillomaviruses, APMIS 118 (2010) 471–493.

[23] L. Ramdas, U. Giri, C.L. Ashorn, K.R. Coombes, A. El-Naggar, K.K. Ang, M.D. Story, miRNA expression profiles in head and neck squamous cell carcinoma and adjacent normal tissue, Head Neck 31 (2009) 642–654.

[24] Q. Rao, H. Zhou, Y. Peng, J. Li, Z. Lin, Aberrant microRNA expression in human cervical carcinomas, Med. Oncol. (2011) [Epub ahead of print].

[25] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative C(T) method, Nat. Protoc. 3 (2008) 1101–1108.

[26] G.K. Smyth, Linear models and empirical bayes methods for assessing differential expression in microarray experiments, Stat. Appl. Genet. Mol. Biol. 3 (2004) Article3.

[27] M. Somberg, S. Schwartz, Multiple ASF/SF2 sites in the human papillomavirus type 16 (HPV-16) E4-coding region promote splicing to the most commonly used 3′-splice site on the HPV-16 genome, J. Virol. 84 (2010) 8219–8230.

[28] D. Spitkovsky, S.P. Hehner, T.G. Hofmann, A. Moller, M.L. Schmitz, The human papillomavirus oncoprotein E7 attenuates NF-kappa B activation by targeting the I kappa B kinase complex, J. Biol. Chem. 277 (2002) 25576–25582.

[29] S.H. Tan, B. Gloss, H.U. Bernard, During negative regulation of the human papillomavirus-16 E6 promoter, the viral E2 protein can displace Sp1 from a proximal promoter element, Nucleic Acids Res. 20 (1992) 251–256.

[30] F. Thierry, P.M. Howley, Functional-analysis of E2-mediated repression of the Hpv18 P105 promoter, New Biologist 3 (1991) 90–100.

[31] K.W. Tsai, C.W. Wu, L.Y. Hu, S.C. Li, Y.L. Liao, C.H. Lai, H.W. Kao, W.L. Fang, K.H. Huang, W.C. Chan, W.C. Lin, Epigenetic regulation of miR-34b and miR-129 expression in gastric cancer, Int. J. Cancer (2011) [Epub ahead of print].

[32] K. Vousden, Interaction of human papillomavirus transforming proteins with the products of tumor suppressor genes, FASEB J. 7 (1993) 872–879.

[33] X. Wang, S. Tang, S.Y. Le, R. Lu, J.S. Rader, C. Meyers, Z.M. Zheng, Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth, PLoS ONE 3 (2008) e2557.

[34] Z.M. Zheng, X. Wang, Regulation of cellular miRNA expression by human papillomaviruses, Biochim. Biophys. Acta (2011) [Epub ahead of print].