Studies on the Stability of Viral and Viral-Cellular Fusion Transcripts Derived from HR-HPV Positive Cervical Carcinomas

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Objective

Virus-DNA integration into the host genome is considered to be one of the key events in HPV-induced cervical carcinogenesis. Several mechanisms by which viral integration contributes to tumour progression are discussed. Jeon and Lambert (Jeon and Lambert, 1995) have mapped instability elements (ARE) in the 3´UTR of the viral oncogene transcripts (figure 1). These elements are generally not retained in viral-cellular fusion transcripts and may therefore enhance mRNA stability and thus yield more oncoprotein. The aim of our study is to provide formal proof for this assumption by comparing the stability of viral early transcripts derived from episomal and integrated HPV16/18 DNA.

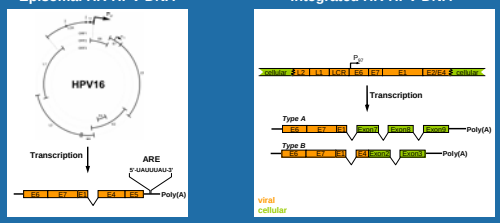

Figure 1: Structural differences between episomal and integrate derived transcripts. Note: Only episomal oncogene transcripts harbour viral A+U rich elements (ARE).

Methods

HPV16/18 positive cervical carcinoma (FIGO stage IA to IIB) were screened for viral-cellular fusion transcripts using the APOT assay (Klaes *et al.*, 1999; Ziegert *et al.*, 2003). Full-length viral-cellular fusion transcripts were amplified by semi-nested PCR and cloned into the adenoviral vector pAD/CMV/V5-Dest™. For control purposes we amplified two of the most abundant early mRNAs derived from episomal viral DNA. These cDNAs comprise the E6-E7-E1VE4-E5 ORF and E6*I-E7-E1VE4-E5 ORF, respectively. All transcripts were amplified with and without the early 3' UTR of the respective HPV genome and mRNA stability is determined after gene transduction into two different mammalian cell lines (C33A and MCF-7) in the presence of Actinomycin-D.

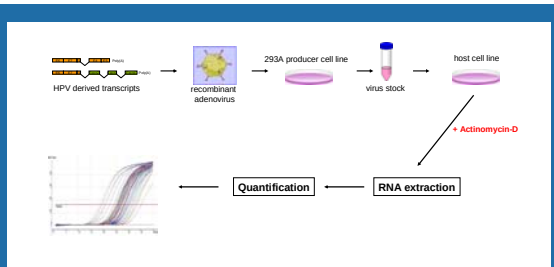

Figure 2: Experimental set up for detection of half-life time of high-risk HPV derived oncogene transcript mRNA/cDNA degradation.

Results

Up to now five cDNAs comprising 5'viral sequences and 3'cellular sequences including the gene specific polyadenylation signal have been successfully cloned in the adenoviral expression vector (table 1). The cellular genes involved are MAP4, SUI1, MGC39325, CDH13 and SLC15A2. All of the required control constructs (viral cDNAs) as well as high titre recombinant adenoviral stocks (10^9 pfu/ml) have been successfully prepared.

Table 1: Summary of the episomal and integrate derived transcripts to study oncogene mRNA/cDNA decay

Transcript	+/- Early 3'UTR	Splicing Pattern	Size [bp]	Cellular Gene Envolved
episomal HPV16 full length	+	83-880v3358-4099	1664	-
episomal HPV16 full length	-	83-880v3358-4099	1551	-
episomal HPV16 E6*I	+	83-226v409-880v3358-4099	1481	-
episomal HPV16 E6*I	-	83-226v409-880v3358-4099	1368	-
episomal HPV16 E6*III	+	83-226v526-880v3358-4099	1010	-
episomal HPV16 E6*III	-	83-226v526-880v3358-4099	897	-
Fusion Transcript T1 MAP4	-	83-226v409-880vExon12vExon13v…Exon19	~2600	Microtubule-associated protein 4
Fusion Transcript T2 SUI1	-	83-226v409-880vExon2vExon3vExon4	~1700	Putative translation initiation factor
Fusion Transcript T3 CDH13	-	83-226v409-880vExon7vExon8v…Exon13	~3700	Cadherin 13, H-cadherin
Fusion Transcript T4 MGC39325	-	83-226v409-880vExon2	~3300	Hypothetical protein MGC39325
Fusion Transcript T5 SLC15A2	-	83-226v409-880vExon20v…Exon22	~1600	Solute carrier family 15 (H+/peptide transporter), member 2

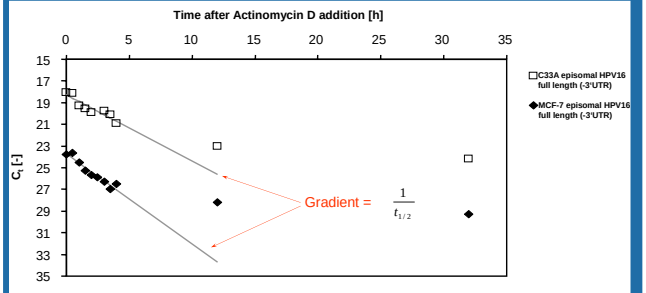

Figure 3: Kinetics of HPV16 full length episomal transcript mRNA/cDNA degradation after addition of transcription inhibitor Actinomycin-D in two different mammalian cell lines (C22A and MCF-7) measured by RT-real time PCR (SYBR green detection; HPV E5 specific primer. Half-life time of the mRNA is calculated from the initial phase of exponential degradation. Note: mRNA concentration is represented by C_t.

Discussion and Ongoing Work

- the above genes of interest can be transduced at high efficiency into host cell lines including primary keratinocytes (>50%) (data not shown).
- first experiments showed that the combination of Actinomycin-D treatment and real-time analysis can be used to determine HPV oncogene transcript decay/stability.
- ongoing experiments aim at validating the previous findings regarding episomal oncogene stability (Jeon and Lambert, 1995) and to compare the results with integrate derived viral cellular fusion transcripts.

References

Jeon, S. and Lambert, P.F. (1995) Integration of human papillomavirus type 16 DNA into the human genome leads to increased stability of E6 and E7 mRNAs: implications for cervical carcinogenesis. *Proc. Natl. Acad. Sci.*, 92, p. 1654-1658

Klaes, R.; Woerner, S.M.; Ridder, R.; Dürst, M.; Schneider, A.; Lotz, B.; Melsheimer, P. and von Knebel Doeberitz, M. (1999) Detection of high-risk cervical intraepithelial neopasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes. *Cancer Res.*, 59, p. 6132-6136

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