ACTIVITY OF LONG CONTROL REGION OF HPV AS DETERMINANT OF ONCOGENICITY

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Introduction

- Human papilloma viruses (HPV) have been associated with benign and malignant disease.
- Approximately 200 HPV genotypes have been described, including:
  - High-risk or carcinogenic viruses such as HPV 16, 18, 31/33/45/52/58 associated with cervical cancers
  - High-risk HPV types associated with head and neck squamous cell carcinomas
  - Low-risk types such as HPV 6 and 11 more frequently associated with benign lesions and aetiological agents of recurrent respiratory papillomatosis (RRP)

Introduction

- The HPV genome is organized into early (E) and late (L) open reading frames (ORFs)
- The genome includes a non-coding region, or long control region (LCR).
- The E6 and E7 genes encode the HPV oncoproteins that promote cell cycle progression and viral DNA replication.
- The LCR contains the viral promoter, p97 and transcriptional elements that regulate expression of the viral oncogenes, as well as transcription factor binding sites and the viral origin of replication.

Introduction

- Our HPV research program includes investigation of HPV types associated with recurrent respiratory papillomatosis (RRP) and HPV associated with head and neck cancers.

Head and neck cancers

- HPV16 and HPV18 account for the majority of HPV-associated HNSCCs,
- During a study to identify HPV genotypes associated with confirmed head and neck cancers, HPV 31 isolate was detected in a laryngeal squamous cell carcinoma.
- HPV16 is the closest relative of HPV31, although HPV 31 is detected less frequently than HPV16
- The complete genome was determined by NGS and the isolate characterised.

Sequence differences in the noncoding LCR relative to the HPV31 prototype sequence (J04353.1).

HPV 31 Prototype nucleotide position	VBD 13/14	Transcription factor binding site
7297–7306 TACTATTTTA	Deletion	-
7314–7323 TTGTTCCTAC[a]	-	-
7338 T	C	
7354 A	G	-
7372 G	C	-
7384 G	A	-
7394 C	A	-
7449–7450 GA	AC	-
7457 G	A	-
7474 C	T	-
7506 C	T	Yin-Yang 1
7525 G	A	-
7575 T	C	-
7710 C	T	-
7754 C	A	-
7865 T	G	-

[a] 10bp deletion is a sequencing error (extra 10 bp insertion) in the prototype genome (J04353) and should not be considered a deletion [8].

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Recurrent respiratory papillomatosis (RRP)

- RRP is caused by HPV infection of the respiratory tract.
- Juvenile onset RRP is a significant public health concern among children in South Africa.
  - Commonly HPV 6 and 11
  - HPV 6 isolates can be divided into 3 variants HPV-6a, 6b and 6vc
  - HPV 11 isolates form two distinct lineages
- We investigated genetic diversity among 31 patients presenting with RRP. Amplification and sequencing of the LCR was done as part of the genome characterization.
- An HPV 6 isolate with a 170 base pair duplication in the LCR was identified in a patient with aggressive disease.

Table 1. *Clinical features of patients with recurrent respiratory papillomatosis*

Laboratory no.	Age at diagnosis (years)	Average Coltera–Derkay score	Total procedures	Average procedures per year while having active disease	Procedures in first year after diagnosis	Average Coltera–Derkay score in first year after diagnosis	Status at last follow-up
2/10	6·3	19·4	15	2·3	4	12·5	Remission
19/10	6·9	26·5	20	5·9	7	26·1	Remission

Results

Table 2. *Schematic representation of the mutations observed within the complete genome of isolates VBD19/10*

	LCR																											
	7349	7356	7400	7452	7467	7513	7514	7515	7516	7517	7518	7519	7520	7616	7617	7618	7623	7628	7661	7696	7700	7747	7748	7812	7840	7884	7929	7954	20
HPV6b	G	C	C	C	G	C	C	T	C	T	T	G	T	T	A	A	C	C	A	T	C	G	C	C	A	C	G	G	T
HPV6a			A	A	T	A		d	d	d	d	d	d	A	C	d	A	T	G	G	G	C	A	I2	C	A		A	I4
VBD19/10			A	A	T	A		d	d	d	d	d	d	A	C	d	A	T	G	G	G	C	A	I2	C	A	I3	A	I4
HPV6vc	T		A	A	T	A	I1								C	C	A	T	G	G	G		A	I2				A	I4

Base positions where mutations were observed are indicated at the top vertically. The shaded blocks indicate no variation from the reference genome. Insertions are indicated by I and deletions are indicated by d.

I1 = TACATTATTGTATA.

I2 = ATATGTTTATTGCCACTGCA.

I3 = TCACCTGGCGCCAGGGTGCGGTATTGCCTTACTCATATGTTTATTGCCACTGCAATAAACCTGTCTTTGTG TTATACTTTTCTGCACTGTAGCCAACTCTTAAAAGCATTTTTGGCTTGTAGCAGAACATTTTTTTGCTCTTACTG TTTGGTATACAATAACATAAAAATG.

I4 = T.

Introduction

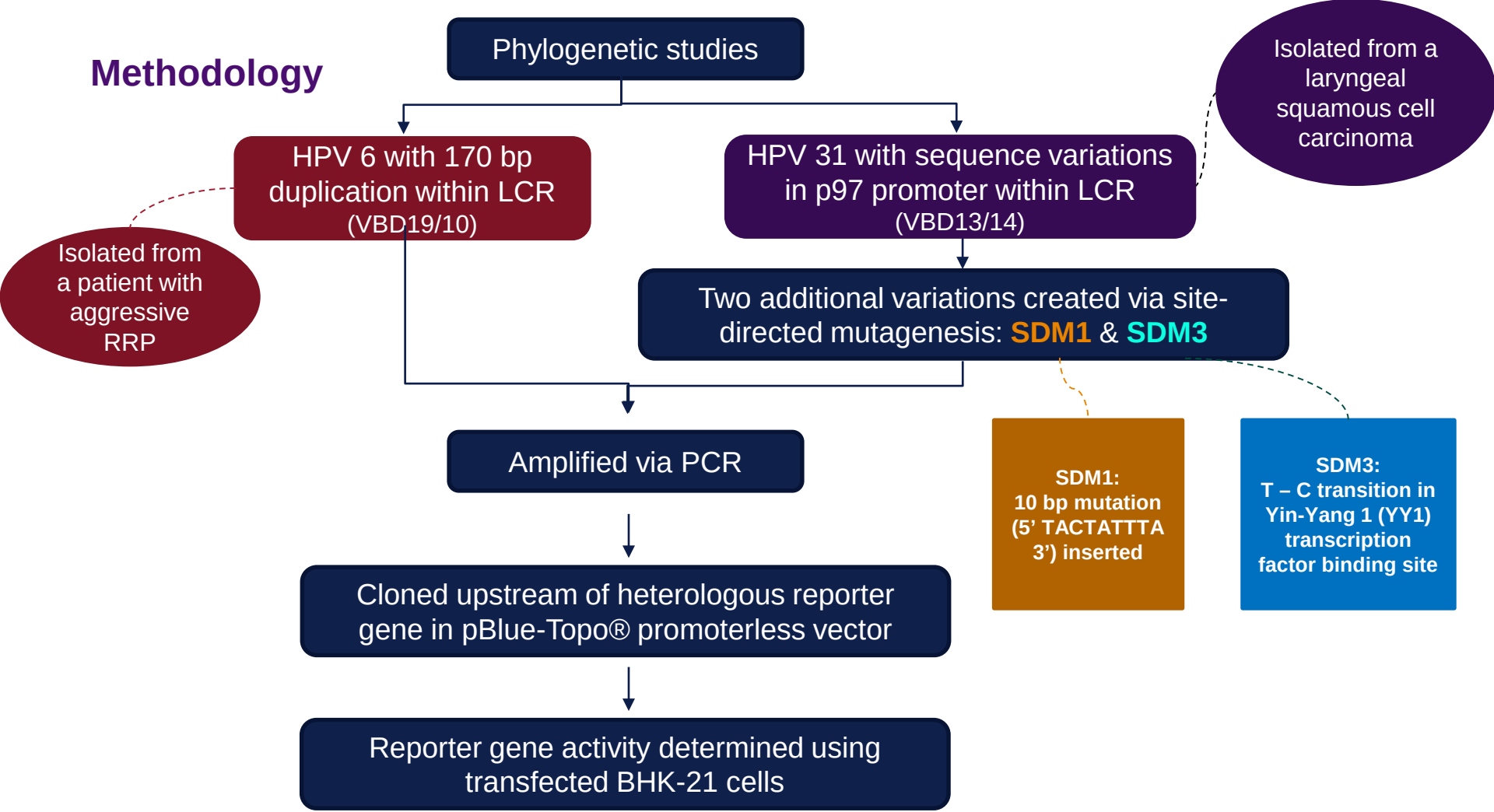
- There is currently no consensus on the influence of genetic mutations on viral replication, pathogenicity and malignant transformations.
- In published reports a duplication of sequence in the LCR from a squamous lung carcinoma was identified and functional assays suggested enhancer functions.
- In contrast other functional assay studies based on differences within the LCR suggested no influence on protein expression.
- It is likely multifactorial
  - Possibly only specific changes may influence expression and pathogenicity
  - Host factors may play a role
- However further studies are warranted to identify possible factors associated with changes in biological/functional activity.

Aim

The influence of nucleotide sequence variations within the HPV genome is not clear and warrants investigation.

To determine if mutations identified in the non-coding LCR of two HPV clinical isolates (type HPV 6 and HPV 31) and deletion in E5 gene had an influence on transcriptional activity using a reporter gene system (*lacZ* which expresses the β-galactosidase enzyme).

Methodology



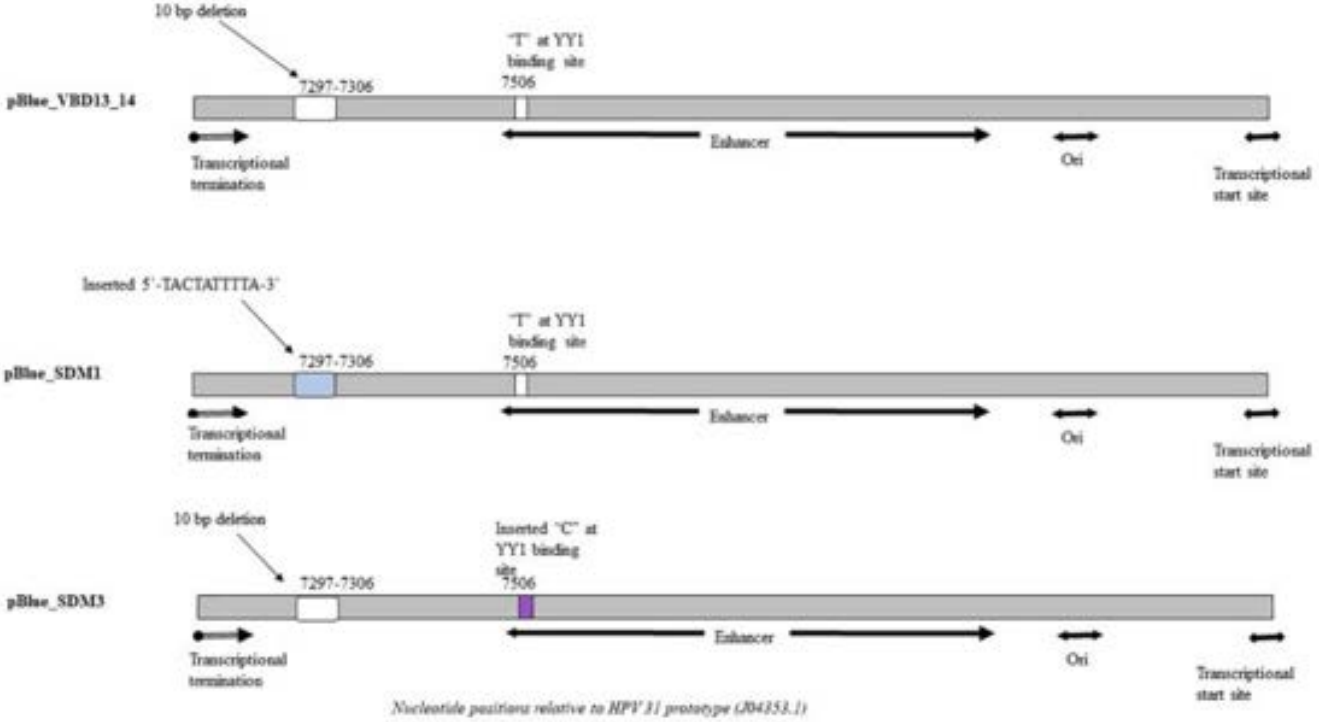


Fig 1. Schematic representation of the full-length LCR variants cloned upstream of the β-galactosidase gene in the reporter vector pBlue-Topo II. YY1 binding site, Yin-Yang 1 binding site; Enhancer, keratinocyte-specific enhancer domain; Ori, origin of replication of the HPV31 circular genome.

Results

Reporter gene activity

HPV 6 with 170 bp duplication within LCR

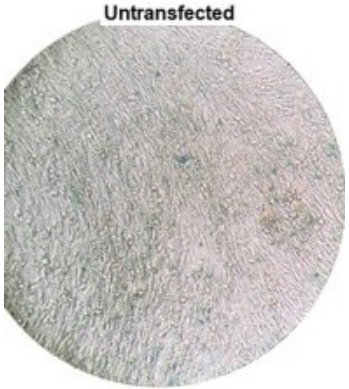
HPV 31 with sequence variations in p97 promoter within LCR

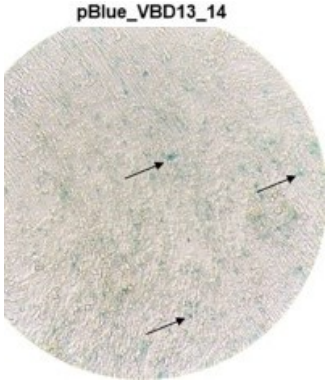
Significantly higher than a control with no duplication (Unfortunately no picture available)

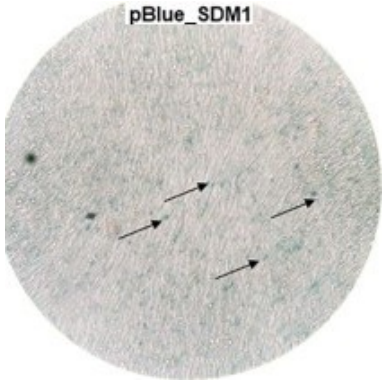
HPV 31 without site-directed mutagenesis (VBD13/14): Enhanced transcriptional activity

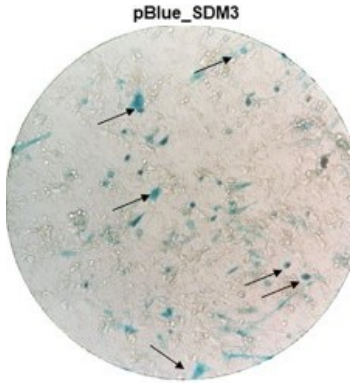
SDM1: No deletion

SDM3: Deletion present and T to C transition in YY1









Results

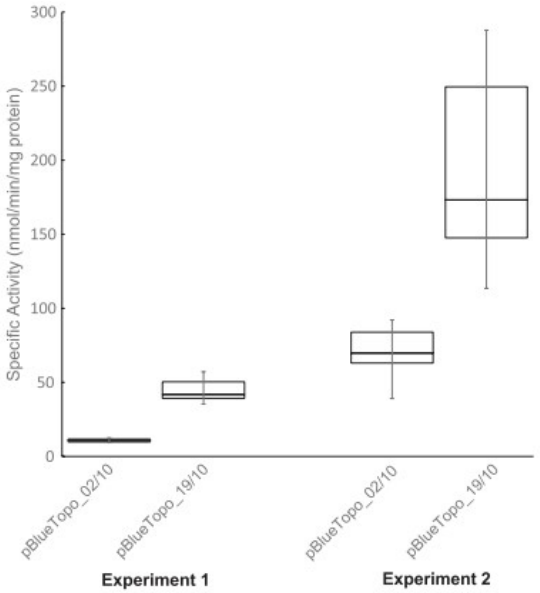


Fig. 2. Level of *β*-galactosidase activity expressed from the *lac*Z gene of cells transfected with the plasmid containing the duplication within the long control region (pBlueTopo_19/10) and without the duplication (pBlueTopo_02/10).

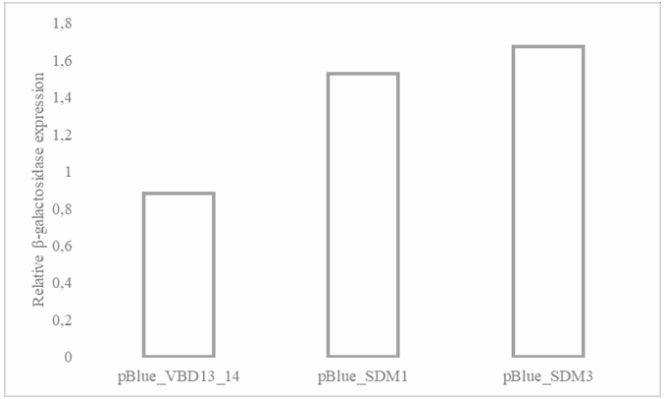


Fig 3. Transcriptional activity of HPV 31 full-length LCR variants. Relative β-galactosidase activities of BHK cells transfected with reporter constructs containing different HPV 31 LCR variants. Data shown represent the means of at least three independent transfection experiments.

Results

- Increased β-galactosidase expression (relative activity) was observed for SDM1 and SDM3 in the absence of the deletion and with the T to C transition
- Hence It was not possible to determine if this was due to the deletion, or the C7506T transition, or other mutations
- Sequence-variation within the LCR of HPV31 isolates may have functional effect on viral p97 promotor activity

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Discussion

- Previous studies suggest that polymorphisms in the LCR may alter the oncogenic potential of the virus.
- Sequence variations relative to the HPV31 prototype sequence were identified.
- The pBlue-Topo® vector, a reporter gene system was used to investigate the possible influence of these variations on the LCR promoter activity in vitro.
- Site-directed mutagenesis used to construct variants with changes that are associated with the prototype sequence (lineage A) allowed for comparison of reporter gene activity.
- Increased β-galactosidase expression was observed for SDM constructs
- The C7506T change occurred at a YY1 binding site. YY1 physically interacts with several proteins regulating cell proliferation and apoptosis
- In certain HPV16 isolates from cervical carcinoma, point mutations or deletions of YY1 binding sites in the LCR were found to have enhanced transcriptional activity.

Discussion

- It is important to note that variation within the LCR is not the only mechanism which leads to HPV types and variants having different oncogenic capacities.
- Natural sequence variation of the HPV31 E6 protein may be involved in the observed differences in the oncogenic potential between HPV31 variants.
- In addition, combinations of amino acid changes with the oncoproteins as well as host factors may also influence oncogenic capacity.
- Further studies could create deletion mutations, to identify specific regions that are responsible for increased transcriptional activity rather than isolating single changes

Discussion

- For the HPV 6 derived LCR the duplication was located where most transcriptionally active binding sites are located.
- Sequence variation within LCR may have a functional effect on viral promoter activity.
- The results suggest that HPV isolates with mutations in the non-coding LCR warrant further investigation for potential biomarkers of aggressive disease.

Conclusion

- HPV 31 results were inconclusive suggesting multiple factors play a role in viral p97 promoter activity
- HPV 6 results suggested that sequence variation within LCR may have a functional effect on viral promoter activity. HPV isolates with mutations in the non-coding LCR warrant further investigation for potential biomarkers of aggressive disease

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Publications from research
Seedat RY, Combrinck CE, Bester PA, Lee J-Y, Burt FJ. (2016) Determination of the complete genome and functional analysis of HPV6 isolate VBD19/10 from a patient with aggressive recurrent respiratory papillomatosis. *Epidemiol Infect* 144: 2128-2135.
Munsamy, Y., Seedat, R.Y., Sekee, T.R., Bester, P.A., Burt, F.J. (2021) Complete genome sequence of a HPV31 isolate from laryngeal squamous cell carcinoma and biological consequences for p97 promoter activity. *PLoS One*. 16(8):e0252524. doi: 10.1371/journal.pone.0252524.

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