Effect of human papillomavirus 16 E6 and E7 oncoproteins on the expression of involucrin in human keratinocytes

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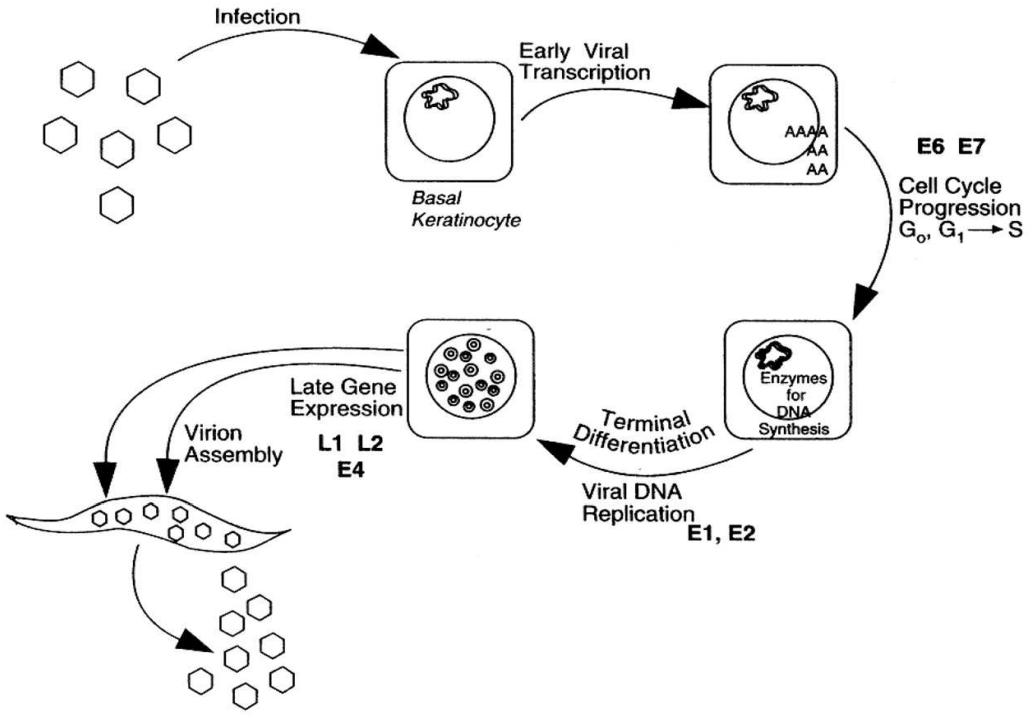
Human papillomaviruses (HPV)

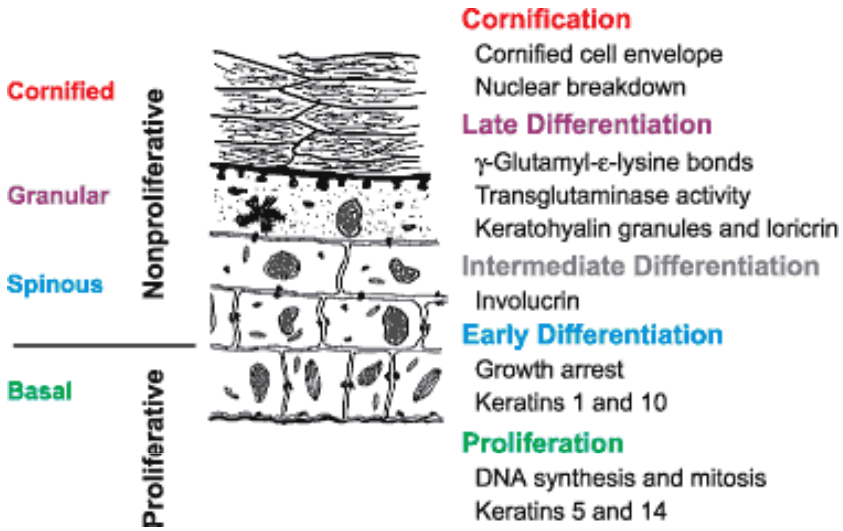
- circular double-stranded DNA genome of about 8 kbp length
- high-risk or oncogenic genital types (HPV 16, 18, and others) are causally linked to the development of cervical cancer
- E6 and E7 oncoproteins of high-risk HPVs are responsible for the transforming activity of the virus
- high-risk HPV E6 induces the degradation of the p53 tumour suppressor protein
- high-risk HPV E7 is able to bind to the pRB (retinoblastoma) tumour suppressor protein
- HPVs infect basal keratinocytes, virus production is associated with terminally differentiated layers



(source: Phelbs et al., *Ann Intern Med.* 1995;123(5):368-382.)

Keratinocyte differentiation and involucrin (IVL)

- during keratinocyte differentiation, the expression of genes involved in the process (such as keratins, transglutaminase 1, involucrin, etc.) is increased
- the expression of involucrin is activated in the spinous layer as a precursor of the keratinocyte cornified envelope
- involucrin is found in the cytoplasm and crosslinked to membrane proteins by transglutaminase



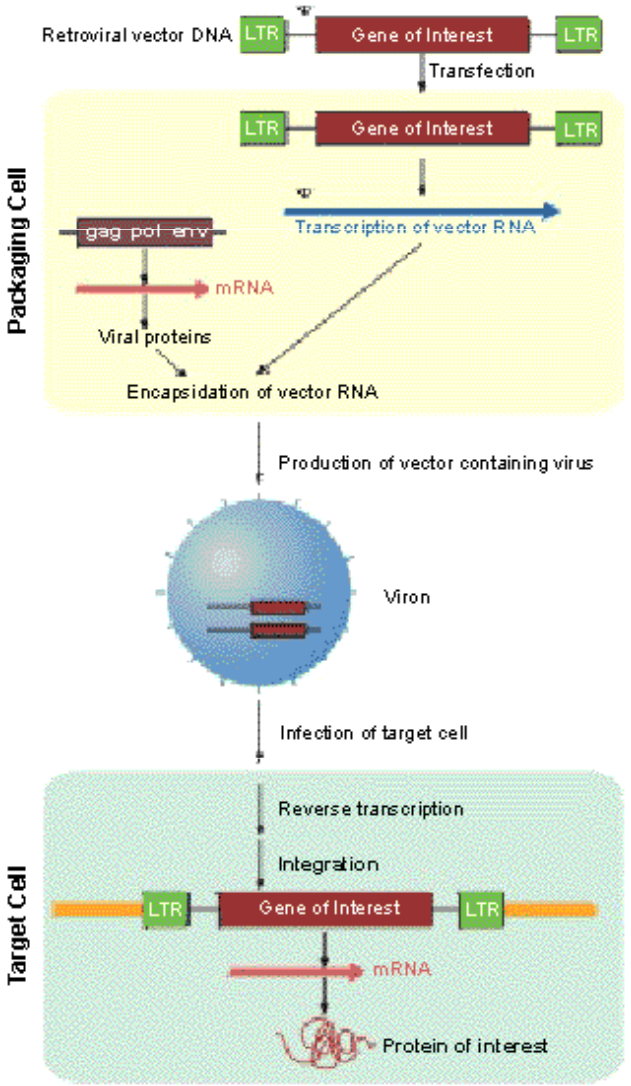
(source: http://quizlet.com/13689178/g2c-integumentary-system-flash-cards/)

Aims

- Investigate the effect of HPV16 oncogenes (E6/E7) and differentiation on the mRNA and protein level of involucrin in human keratinocytes.
- Study in transient tranfection assays the effect of HPV E6 and E7 on the activity of involucrin promoter.

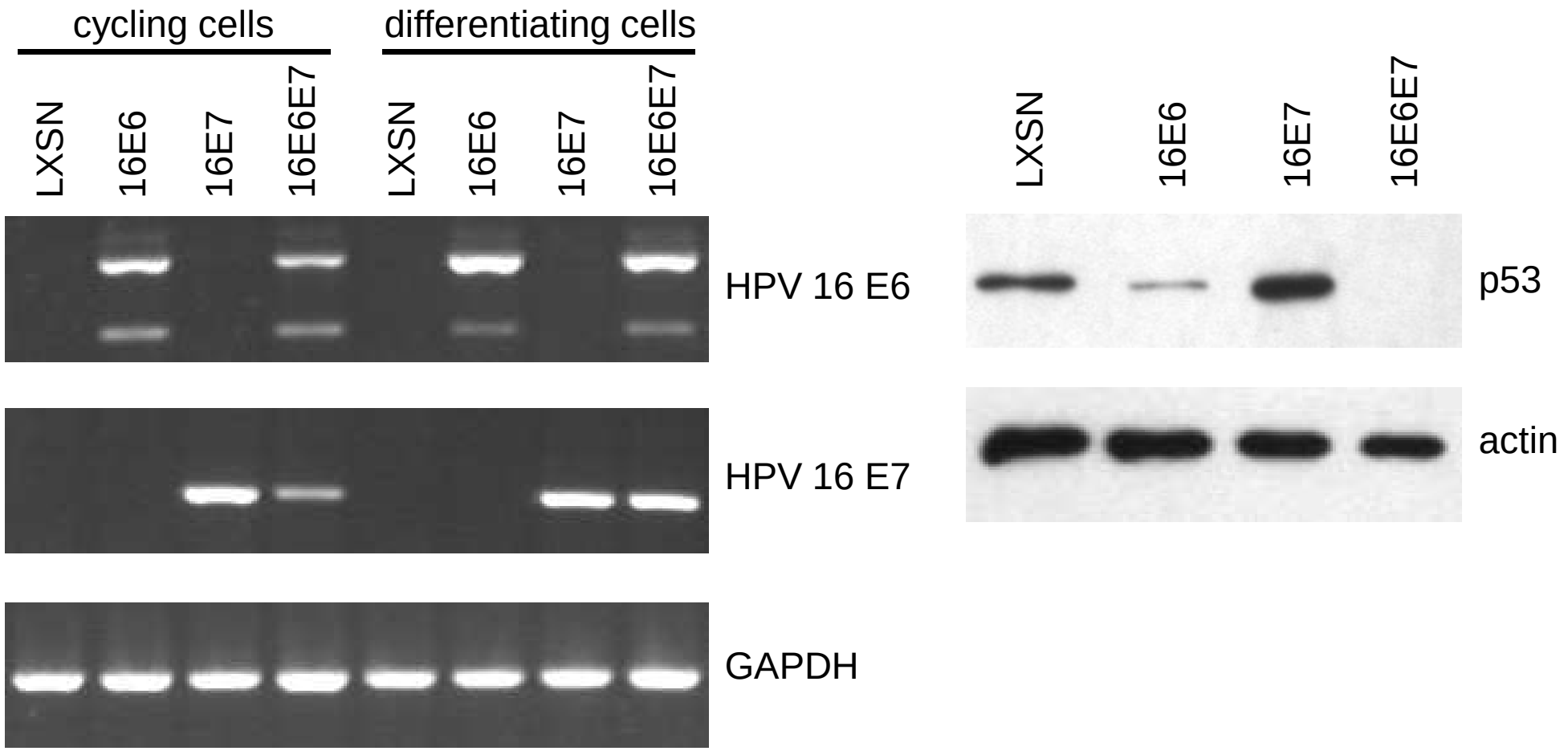
Materials and methods

- ***Cell culture and viral transduction***:
  - primary human foreskin keratinocytes (HFK) in serum free, low calcium medium
  - transduction by LXSN retrovirus expressing HPV16 E6, HPV16 E7 or HPV16 E6/E7 genes
  - induced to differentiate by culture in DMEM (high calcium and serum) for 24h
- ***Real-time PCR***:
  - total RNA isolation
  - reverse transcription
  - real-time PCR: TaqMan Assay for involucrin and GAPDH as endogen control
- ***Western blot***:
  - monoclonal anti-human involucrin antibody
- ***Transient transfection***:
  - Effectene transfection reagent
  - reporter vector (pGL3): luciferase reporter carrying different fragments of involucrin promoter
  - expression vectors (pcDNA 3.1): pcDNA-16E6, pcDNA-16E7
  - After transfection, cells were either left untreated or induced to differentiate
  - Luciferase assay, standardized by Bradford protein assay

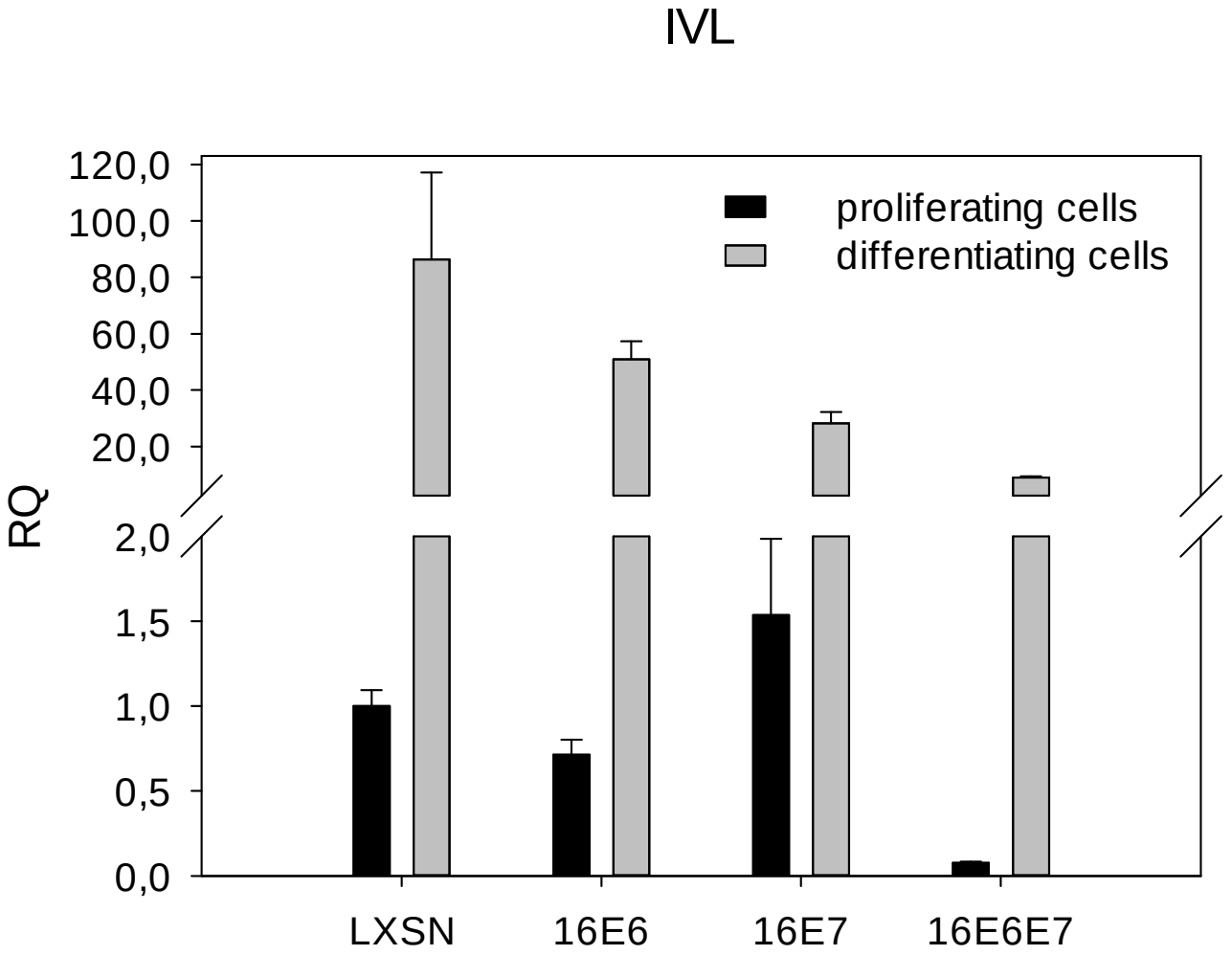


(source:www.biocompare.com/Articles/ApplicationNote/180/High-Titer-Retroviral-Vectors-For-Gene-Delivery.html)

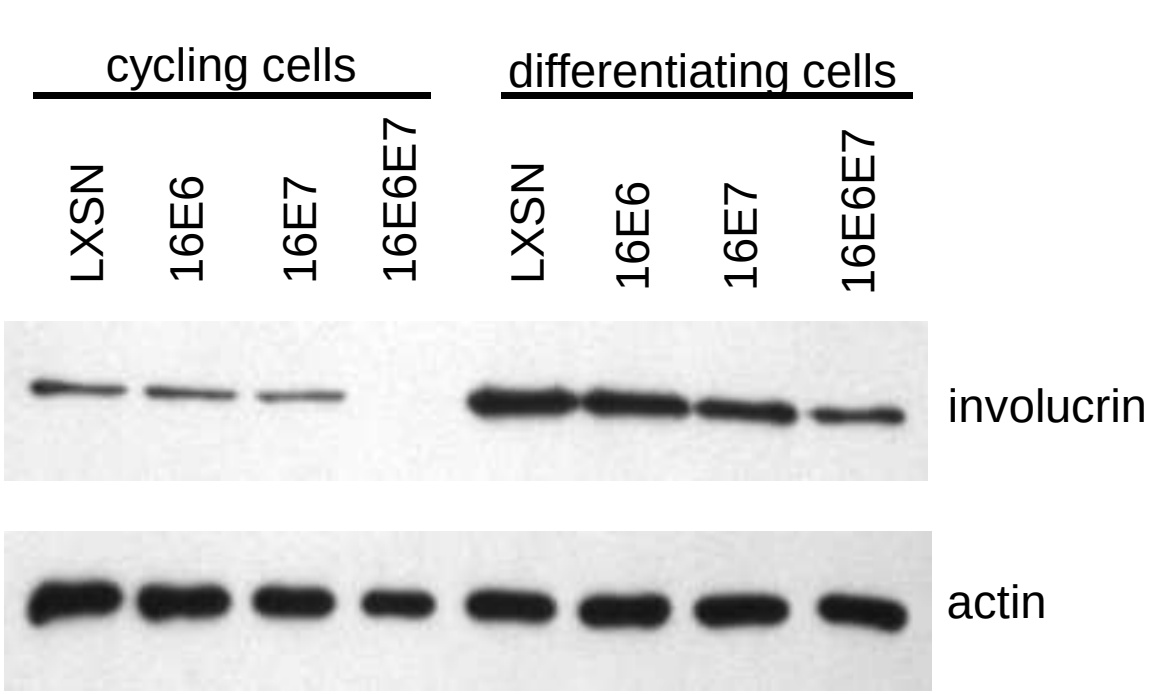
Generation of HFK cells expressing HPV 16 oncogenes

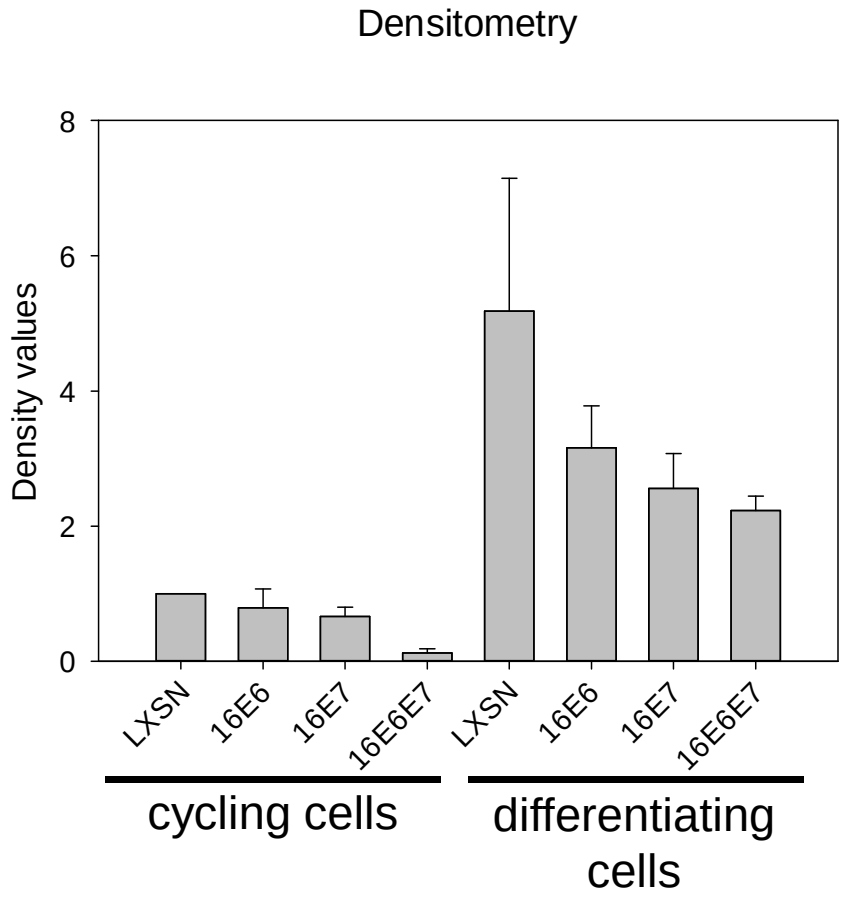


Real-time PCR

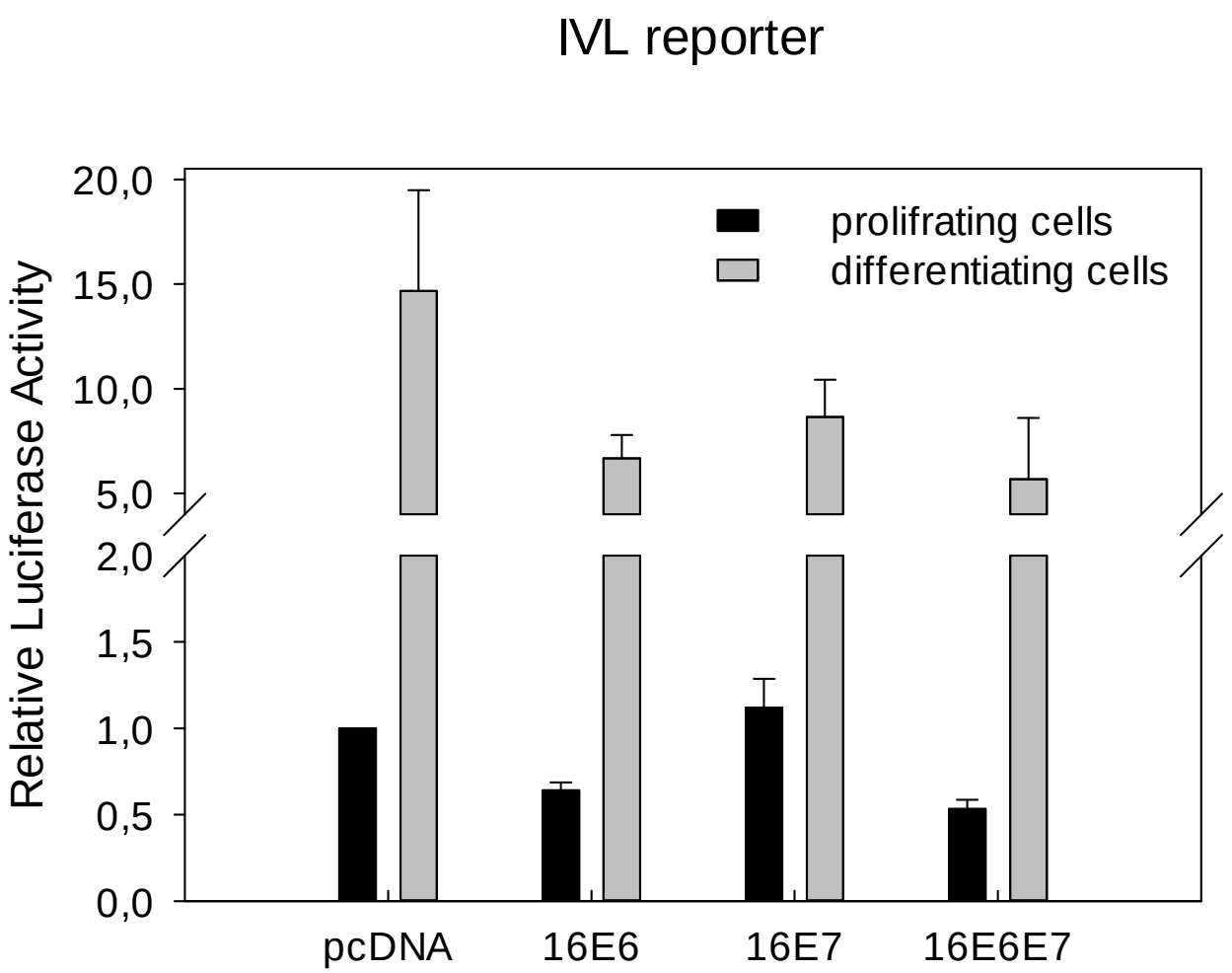


Western blot

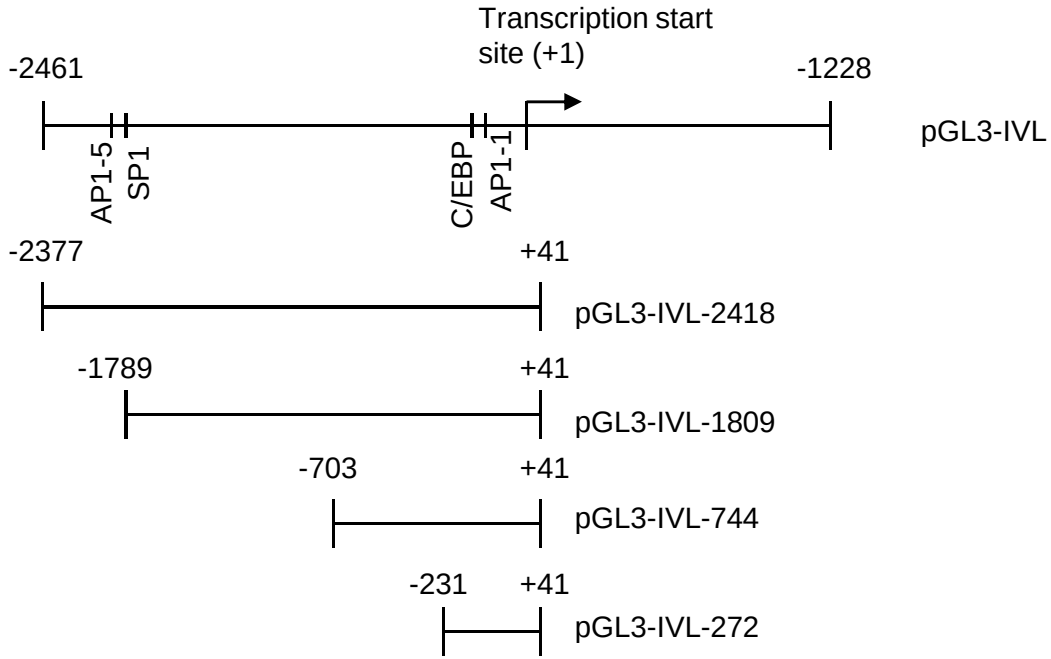


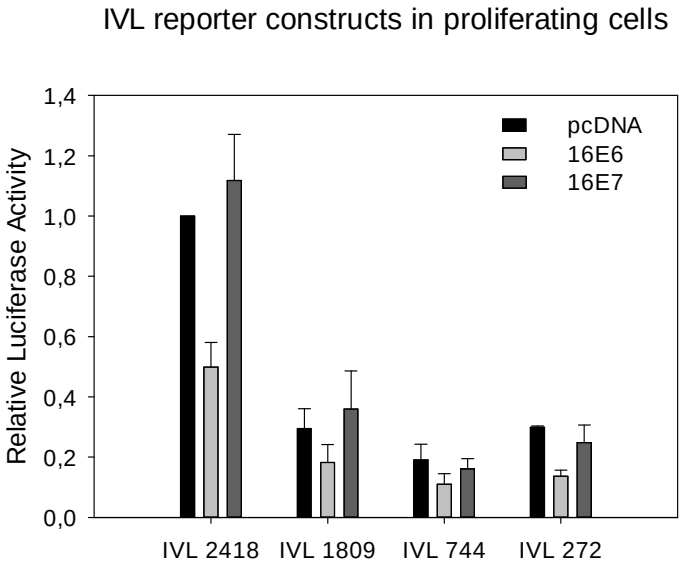


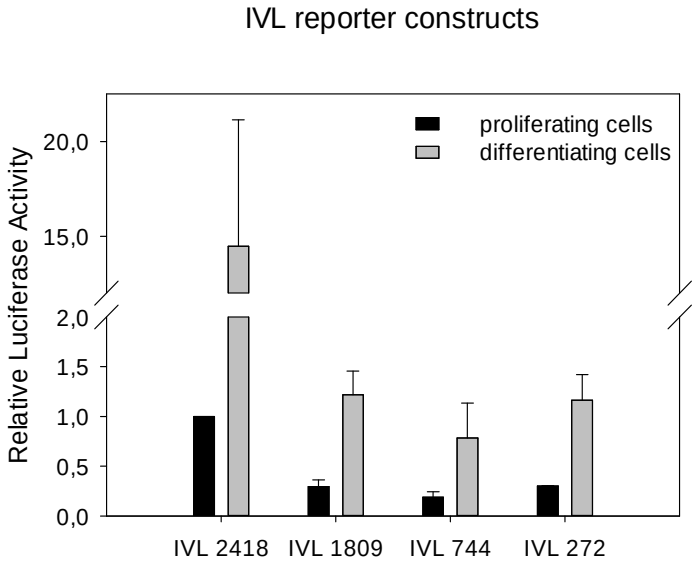
Transient transfection

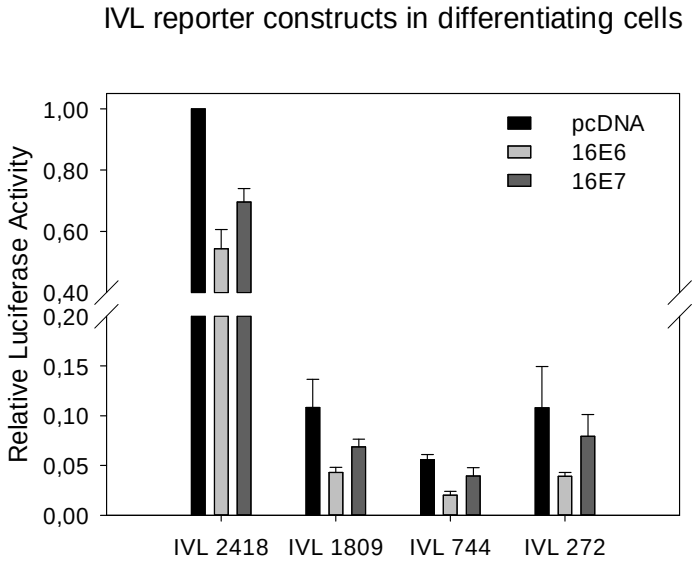


Localization of the effects of HPV 16 oncogenes on IVL promoter









Conclusions

- The differentiation of keratinocytes by serum and calcium highly increased both the mRNA and the protein levels of involucrin.
- The E6 and E7 oncogenes of HPV16 together caused downregulation of the involucrin mRNA and protein both in proliferating and differentiating cells.
- In transient transfection assays the HPV E6 repressed involucrin promoter activity in proliferating cells and both HPV oncoproteins caused a down-regulation of the promoter activity in differentiating cells.
- The effect of HPV E6 was localized to the proximal region of the involucrin promoter.