The potential of RNA as a target for molecular diagnostics in cervical carcinoma screening.

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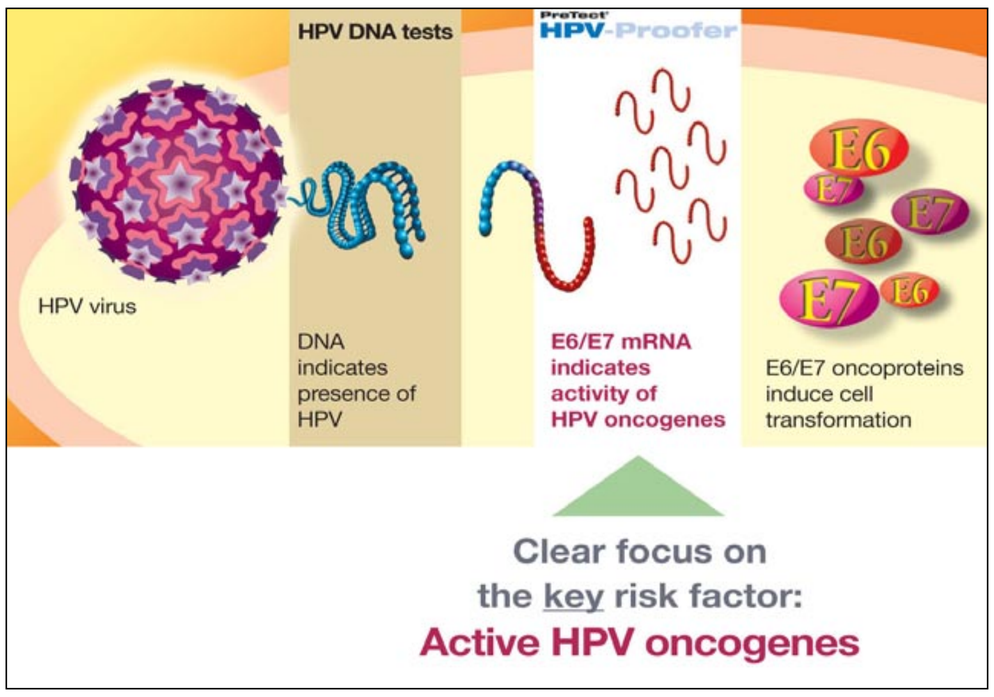
Introduction

Microarray technology and varied multiplex amplification methods (e.g. PreTect technology) have shown that RNA is valid as a target for routine molecular diagnostics and for future point-of-care testing. Detection of human papillomavirus targets may lend more accuracy and objectivity to the diagnostic process but consensus DNA-HPV detection methods have to be replaced with type-specific PCR and/or HPV RNA based detection methods, in order to increase the clinical specificity and positive predictive value (Table 2).

The association between E6-E7 gene products and cervical carcinogenesis may be the strongest ever-discovered including complex interactions with various cellular protein complexes, cell senescence and severe chromosomal instability (Figure 2) (von Knebel et al 2002). The main challenge using protein as a target for routine diagnostics has been low sensitivity, reproducibility and specificity. However, RNA and not DNA as a target for routine diagnostics may give the information of clinical activity, regulation or processes in addition to higher or equal sensitivity, reproducibility and specificity compared to DNA as target (Table 2). For more than 10 years ago new methods of isolation, purification and stabilization of mRNA was developed for routine diagnostics making the RNA very much suited as a marker for development of new diagnostics methods and even drugs. Available evidence indicated great potential for HPV testing within the cervical screening program. It offers the possibilities of greater sensitivity, reduced follow-up of low grade cytological abnormalities and treated lesions, increased screening intervals, and overall cost reduction (Cuzick 2002). An attractive screening option is to use HPV RNA and typing of a limited number of HPV types as the primary test and reserve cytology for triage to determine management.

Fig 1: HPV-Proofer detect E6/E7 mRNA from the five carcinogenic HPV types indicating activity of HPV oncogenes.



HPV-Proofer correlated with other methods

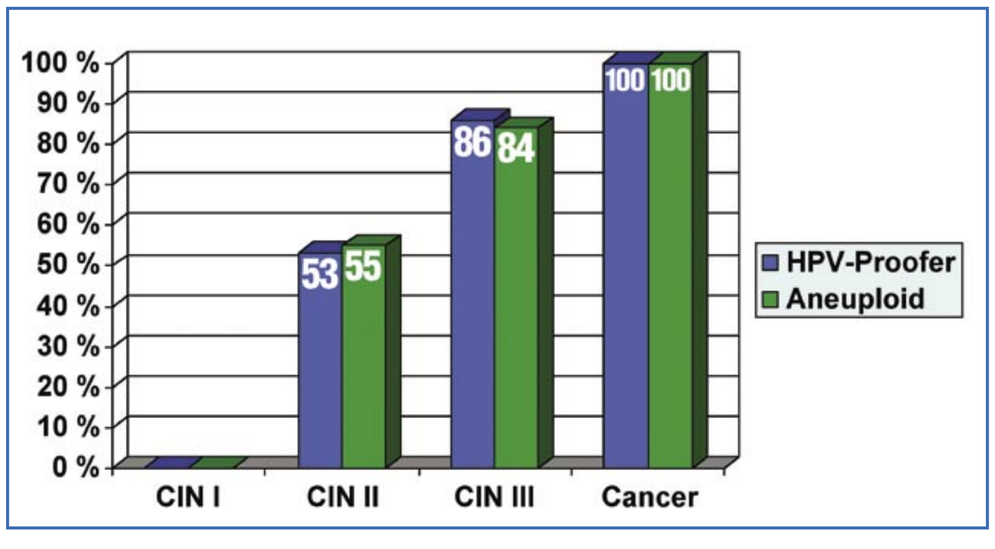
The HPV-Proofer method was developed to perform typing of HPV types 16, 18, 31, 33 and 45 with concurrent detection of expression markers (bicistronic mRNA of known oncogenes E6 and E7) in order to detect loss of viral and cell cycles regulation presented as a major cell nuclei abnormality. HPV-Proofer was evaluated against the following methods in the below listed clinical studies.

- Type-specific PCR
- Quantitative real-time PCR
- Consensus PCR
- HCII
- In situ hybridization
- Line-blot PCR
- NASBA with end-point ECL detection
- Singleplex real-time NASBA
- Sequencing analysis

Clinical studies

1. E6 and E7 mRNA expression in 4136 clinical samples (Molden, 2004 Poster number)
2. Development and verification of HPV-Proofer (Molden, 2003, submitted 2004)
3. High risk infections without oncogene expression in cervical smears (Molden 2004, Poster)
4. Cancer study on 204 squamous carcinoma samples (Lie, 2004, in prep 2004)
5. CIN III study on 190 biopsies (Kraus, BJC, in press 2004)
6. Follow up study in Edinburgh (Cuschieri, JMV, in press 2004, Poster)
7. Validation study in Oslo (Lie, 2004, Poster and Oral presentation)
8. Karlstad follow-up study in Sweden (Bjerre, 2003-2004, Poster)
9. Out-patience population study in DR Congo (Hovland, 2004)
10. High risk oncogene expression in progressive lesions (Nygaard, 2004, Poster and Oral pres.)
11. Evaluation of the main transcripts in clinical samples (Molden, in prep 2004)
12. Evaluation of the stability of mRNA from HPV 18 and 45 (Kraus, in prep 2004)

Fig 2:
Rate of mRNA expression in two clinical studies and DNA ploidy aberrations in biopsies (Skyldberg et al., 1999, Molden, 2004, Kraus, 2004, Lie et al., in prep).



This is two of our studies in blue compared with cytometry results done by Barbro Skyldberg at Karolinska Institutes

Table 1: mRNA and DNA analysis in 4136 cervical smears collected in a screening population in Norway.

	Normal	ASCUS	Condyloma	CIN I	CIN II	CIN III	SCC	Total
HPV mRNA	95 2%	12 21%	6 32%	0 0%	2 40%	9 75%	1 100%	126 3%
HPV DNA	368 9%	27 47%	14 74%	1 100%	2 40%	10 83%	1 100%	429 10%
Cytology	3970	57	19	1	5	12	1	4136*

*Results from groups unsatisfactory (64), AGUS (5) and ASC-H (2) not shown

Conclusion

1. Incidence of HPV oncogene expression increases with the severity of the lesion. **2.** Improved clinical specificity for HPV RNA compared to HPV DNA in "normal" and "ASCUS" samples. **3.** Detecting HPV oncogenic activity in combination with typing promises to be a powerful predictor of high-grade lesions. **4.** All of the cytological cell samples collected from women with cervical carcinoma were positive with HPV-Proofer (n=20). **5.** The significant majority of cancers (>96%) contain at least one of the five main carcinogenic HPV-types (HPV 16, 18, 31, 33 and 45). **6.** Those who tested positive with HPV-Proofer were significantly more likely to maintain a persistent infection than those without positive HPV-Proofer results. **7.** HPV-Proofer in combination with cytology gives the maximum security for physicians and patients (~100% sensitivity/specificity).

Table 2:
Prevalence, sensitivity and specificity in four large clinical studies

	Histology and Cytology	Molecular biology	Sensitivity	Specificity	Relative PPV	Relative NPV
Validation study (>30 years)	339 cyt norm. 288 cyt pos. 61 hist norm. 322 hist pos.	HPV-Proofer	76%	81%		
		HCII	93%	40%		
Validation study (<30 years)		HPV-Proofer	82%	70%		
		HCII	98%	20%		
Oslo study (n=4136)	3970 cyt norm. 95 cyt pos. 25 hist pos.	HPV-Proofer	86%	97%	7%	100%
		PCR consensus	93%	90%	2%	100%
Edinburgh study (follow-up) (n=3445)	3445 cyt norm. 54 PCR pos.	HPV-Proofer	100%	78%	27%	100%
		PCR consensus	100%	40%	12%	100%
Karlstad study (follow up) (n=15000)	127 cyt norm. 90 cyt pos. 18 hist norm. 100 hist pos.	HPV-Proofer	81%	85%	46%	95%
		HCII	91%	51%	31%	96%

References

von Knebel el al., Eur J Cancer 2002, 38: 2229-42; Clifford et al., BJC 2003, 88:63-73;