RECOMBINANT E6 ONCOPROTEINS OF DIFFERENT HUMAN PAPILLOMAVIRUSES: NOVEL TOOLS FOR HPV TUMOR DIAGNOSIS

Elena Illiano [a], Rosella Franconi [b], Antonio Rinaldi [a]

[a] NANOFABER s.r.l , Via G.A. Badoero 82, 00154, Rome, Italy
[b] ENEA, Casaccia Research Center, Department for Sustainability, Via Anguillarese 301, 00123, Rome, Italy
e-mail: elena.illiano@nanofaber.com

Keywords: (HPV, E6 oncoprotein, Biomarker, Diagnostic)

High-risk human papillomaviruses (HR-HPVs) types 16 and 18 are the main etiological agents of cervical cancer, with more than 550.000 new cases each year worldwide. HPV16 and HPV18 have also been shown to cause almost half the vaginal, vulvar, and penile cancers, while about 85% of anal cancers are also caused by HPV16 [1]. HPVs, and HPV16 in particular, are associated with some head and neck squamous-cell carcinomas, and they are an independent risk factor for oropharyngeal cancers [2].
The HR-HPV E6 and E7 oncoproteins are responsible for onset and maintenance of the cell transformation state, and they represent appropriate targets for the development of new diagnostics.
Moreover, in the last years, HPV16 E6 serology was identified as a promising pre-diagnostic marker for HPV-driven cancers, as HPV16 E6 seropositivity has been found more than 10 years before diagnosis of oropharyngeal cancers [3]. It is also important to note that seropositivity is relatively common before diagnosis of anal cancer, although it is rare for other HPV-related ano-genital tumors [4].
Recombinant E6 protein is extremely difficult to obtain in a soluble and active form. We set up a protocol for the production of soluble Histidine-tagged E6 protein (His_6-E6), from HPV-16, -18, -11, in native conditions from bacteria. [5].The structural properties of HPV16 His_6-E6 were determined using circular dichroism and fluorescence spectroscopy and suggest that the protein maintains correct folding. His_6-E6 oncoprotein immunogenicity was assessed in a mouse model showing a significant humoral immune response. The E6 proteins from HPV16, HPV18, and HPV11 were purified according to a new procedure and investigated for protein–protein interactions. Its functionality was determined using *in vitro* GST pull-down and protein degradation assays. HR-HPV His_6-E6 bound p53, the PDZ1 motif from MAGI-1 proteins, the human discs large tumor suppressor (hDLG) and the human ubiquitin ligase E6-associated protein (E6AP), thus suggesting that they are biologically active. The purified HR-HPV E6 proteins also targeted the MAGI-3 and p53 proteins for degradation. Moreover, we demonstrated that our HPV-16 E6 protein is stable at + 4 °C for at least 2 years [6].
This new procedure can be useful to prepare the E6 protein to promote its industrial production for diagnostic tests. With this aim, the maintenance of the native conformation of the E6 protein should improve the specificity, costs, precision, and reproducibility in the detection of anti-E6 antibodies in patient sera.
Our goal is the development of a simple,rapid,reliable,portable,low-cost diagnostic kit, based on direct/indirect detection of E6 biomarker, which is expressed at high levels in samples only when HPV-infected cells undergo precancerous and cancerous changes.
Ongoing experiments to immobilize HPV E6 proteins on chips based on elettrospun biopolymers, for the detection of serum antibodies in patients, will be reported.

[1] D.P. Zandberg *et al*., Cancer J Clin. (2013) 57–81
[2] M.J. Worsham *et al*., Mol Diagn Ther. (2012) 209–22
[3] A.R. Kreimer *et al*., J Clin Oncol. (2013) 2708–15
[4] A.R. Kreimer *et al*., J Clin Oncol. (2015) 877–84
[5] R. Franconi & E. Illiano (2007) Brevetto Italiano n. 1379103
[6] Illiano *et al*. J Transl Med (2016) 14:224

Protocol	Temperature (°C)	Dithiothreitol (μM)	pH	Imidazole (mM)			Detergent[a]
				Lysis buffer	Wash buffer	Elution buffer	
A	Room temperature	–	8	10	20	250	–
B	4	100	8	10	25	250	–
C	4	100	8	20	50	250	–
D	4	100	7.5	20	70	300	–
E	4	100	7.5	20	70	300	0.02 % lauryl-β-D-maltoside 0.02 % Tween-20 0.02 % glycine 0.02 % betaine 0.1 M arginine
F	4	100	7.5	20	70	300	0.02 % betaine

[a] The detergents listed were tested individually

Figure 1. Table of Chemical and physical parameters analyzed during the purification of the HPV16 His_6-E6 protein under native conditions

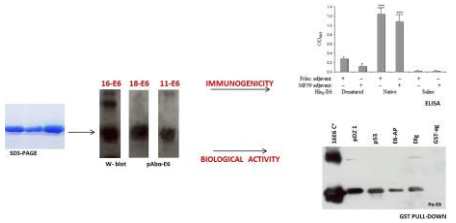

Figure 2. Purification of the His_6-E6 proteins from HPV16 18 and 11 under native conditions. Anti-E6 humoral responses in mice. Physical interactions between the purified HPV16 His_6-E6 protein and its cellular targets.

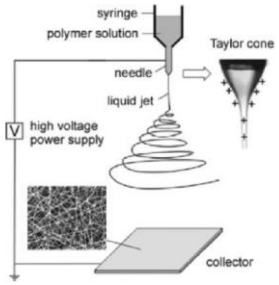

Figure 3. Schematics of electrospinning setup

Figure 4. Prototype chip for detection of serum antibodies in patiens