

POSTER PRESENTATION

Open Access

# Transient expression of the cancer/testis cancer antigen NY-ESO-1 in *Nicotiana benthamiana* using a PVX-based viral vector

Cristiano Lacorte<sup>1\*</sup>, Jéssica Carrijo<sup>2</sup>, André Murad<sup>1</sup>, Elíbio Rech<sup>1</sup>

From 5th Congress of the Brazilian Biotechnology Society (SBBIOTEC)  
Florianópolis, Brazil. 10-14 November 2013

## Background

Cancer/testis antigens is a group of proteins which expression is usually restricted to the human germ line but is also present at high levels in several types of cancer cells [1]. The antigen NY-ESO-1 is one of these antigens showing high immunogenicity and, therefore, holding great potential for a cancer vaccine [2]. Its heterologous expression is being tested in different expression systems, although the success has been limited [2,3]. In this report we used a transient expression system based on a PVX viral vector to evaluate the expression of NY-ESO-1 in leaves of *Nicotiana benthamiana*.

## Methods

The NY-ESO-1 antigen coding sequence was synthesized based on a plant codon optimized sequence. The correspondent fragment was cloned in a pDonr207 derived vector containing a hexa-histidine tag (6His) and signal peptides for the endoplasmic reticulum (ER), apoplast or chloroplast. These vectors were recombined to a Gateway<sup>®</sup>-compatible PVX-based vector using the LR clonase, and transferred to *Agrobacterium tumefaciens* strain GV3101 containing the pSoup vector [4]. *Nicotiana benthamiana* plants were inoculated with *Agrobacterium* suspensions through vacuum infiltration. After 4-5 days, leaves were collected and macerated in phosphate saline buffer (PBS) at a 1:2 (w:v) ratio and analyzed by Western blot.

To further characterize and quantify the expression levels of recombinant NY-ESO-1, leaf extracts were analyzed by nanoUPLC-MS<sup>E</sup> [5]. To this end, leaves were grinded and proteins were extracted using 50mM

Tris-HCl pH 8.0 in 1:20 (w:v) ratio. Proteins were precipitated with acetone, resuspended in water and quantified using the Qubit<sup>®</sup> fluorimetric assay.

## Results and conclusions

The expression of cancer/testis NY-ESO-1 antigen was detected in *N. benthamiana* leaves using a PVX-based transient assay. Different constructs were tested containing signal peptides for ER, apoplast and chloroplast. No signal was detected when the NY-ESO-1 antigen was targeted to chloroplast or to the apoplast and detection was only possible when the protein was targeted to the ER, indicating that NY-ESO-1 accumulation in leaf tissue is influenced by the subcellular localization. NanoUPLC-MS<sup>E</sup> analysis confirmed the presence of heterologous NY-ESO-1 in a level of approximately 0.1 % of the total soluble protein extract.

This assay allows protein to be detected after only 4-5 days after inoculation thereby eliminating the need of long and cumbersome tissue culture and selection schemes necessary for transgenic plants production. It also represents a handy and fast method to evaluate different gene constructs.

## Acknowledgements

FAP-DF, CAPES, CNPq, Embrapa.

## Authors' details

<sup>1</sup>Embrapa Recursos Genéticos e Biotecnologia, Estação Parque Biológica, 70770-910, Brasília, DF, Brazil. <sup>2</sup>Centro de Análises Proteômicas e Bioquímicas, Universidade Católica de Brasília, Brasília, DF, Brazil.

Published: 1 October 2014

## References

1. Caballero OL, Chen YT: **Cancer/testis (CT) antigens: potential targets for immunotherapy.** *Cancer science* 2009, **100**(11):2014-2021.

<sup>1</sup>Embrapa Recursos Genéticos e Biotecnologia, Estação Parque Biológica, 70770-910, Brasília, DF, Brazil

Full list of author information is available at the end of the article

2. Simpson AJ, Caballero OL, Jungbluth A, Chen YT, Old LJ: **Cancer/testis antigens, gametogenesis and cancer.** *Nature reviews* 2005, **5**(8):615-625.
3. Lowe AJ, Bardliving CL, Huang CJ, Teixeira LM, Damasceno LM, Anderson KA, Ritter G, Old LJ, Batt CA: **Expression and purification of cGMP grade NY-ESO-1 for clinical trials.** *Biotechnology progress* 2011, **27**(2):435-441.
4. Lacorte C, Ribeiro SG, Lohuis D, Goldbach R, Prins M: **Potatovirus x and Tobacco mosaic virus-based vectors compatible with the Gateway cloning system.** *Journal of virological methods* 2010, **164**(1-2):7-13.
5. Murad AM, Souza GH, Garcia JS, Rech EL: **Detection and expression analysis of recombinant proteins in plant-derived complex mixtures using nanoUPLC-MS(E).** *Journal of separation science* 2011, **34**:2618-2630.

doi:10.1186/1753-6561-8-S4-P76

**Cite this article as:** Lacorte *et al.*: Transient expression of the cancer/testis cancer antigen NY-ESO-1 in *Nicotiana benthamiana* using a PVX-based viral vector. *BMC Proceedings* 2014 **8**(Suppl 4):P76.

**Submit your next manuscript to BioMed Central  
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
[www.biomedcentral.com/submit](http://www.biomedcentral.com/submit)

