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

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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 benthamina*.

Methods

The NY-ESO-1 antigen coding sequence was synthetized 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®-compatible PVX-based vector using the LR clonase, and transferred to *Agrobacerium tumefaciens* strain GV3101 containing the pSoup vector [4]. *Nicotiana benthamina* 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^E [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® 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^E 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.

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