STSM Final Report

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Host: Dr. Pilar Alberdi and Professor José de la Fuente, Instituto de Investigación en Recursos Cinegéticos (IREC), Ciudad Real, Spain
Period of Stay: From 6th to 18th of December 2015

Report

Improving current understanding and research for sustainable control of the poultry red mite Dermanyssus gallinae (COREMI)

STSM title: Silencing of target genes in tick cell lines in vitro to further assess their potential function to reduce the population of vectors namely mite

Description of the work: According to the proposed working plan, based on the Working Group 1 of COREMI, silencing of target genes in tick cell lines in vitro was performed, to further assess their potential function to reduce the population of vectors. Under the supervision of Dr. Pilar Alberdi and Professor José de la Fuente, from the 6th until the 18th of December of 2015, tick cell cultures were maintained to further silencing assays at the Instituto de Investigación en Recursos Cinegéticos (IREC), in Ciudad Real (Spain).

Two strains of tick cell lines from *Ixodes scapularis*, ISE6 and IDE8, were maintained and cultured in L15B300 and L15B medium, respectively[1]. Double-stranded RNA (dsRNA) of 2 genes were used to evaluate their function and *I.scapularis* unrelated Rs86[1] dsRNA was used as negative control. In a 24-wellplate, were used four conditions in triplicate: without dsRNA as a control, with dsRNA from Rs86 gene as a negative control and AP1 and AP2 as target genes. A solution of 10µL of dsRNA within $5x10^{10}$ to$5x10^{11}$ molecules/µl and 90µl of respective medium, was incubated with ISE6 and IDE8 tick cells for four days. After RNAi exposure, tick cell lines were collected to perform viability assays and stored for further RNA and DNA extractions. To detect cell viability after silencing assays, apoptosis was measured by flow cytometry using Annexin V - FITC apoptosis detection kit (Immunostep, Salamanca, Spain)[1]. Since apoptosis facilities the exposure of phosphatidylserine, annexin labelled with FITC binds. Besides, propidium iodide (PI) is also added to stain dead cells. The objective of this experiment is to analyse apoptotic cells with Annexin V – FITC positive, PI negative). All samples were analyzed on a FAC-Scalibur flow cytometer (BD Bio- sciences, Madrid, Spain), considering viable cells acoording to

forward-scatter and side-scatter parameters.

The percentage of apoptotic cells was determined by flow cytometry and compared between different conditions and the control without dsRNA, using one-way ANOVA (p<0.05).

Description of the main results: Results indicate that knockdown of selected genes do not influence significantly ISE6 and IDE8 cells (Figure 1A e B). Silencing of these genes do not interfere with apoptosis.

Further analysis will be conducted in Instituto de Higiene e Medicina Tropical (IHMT) to evaluate the percentage of gene silencing by qPCR.

A **B**

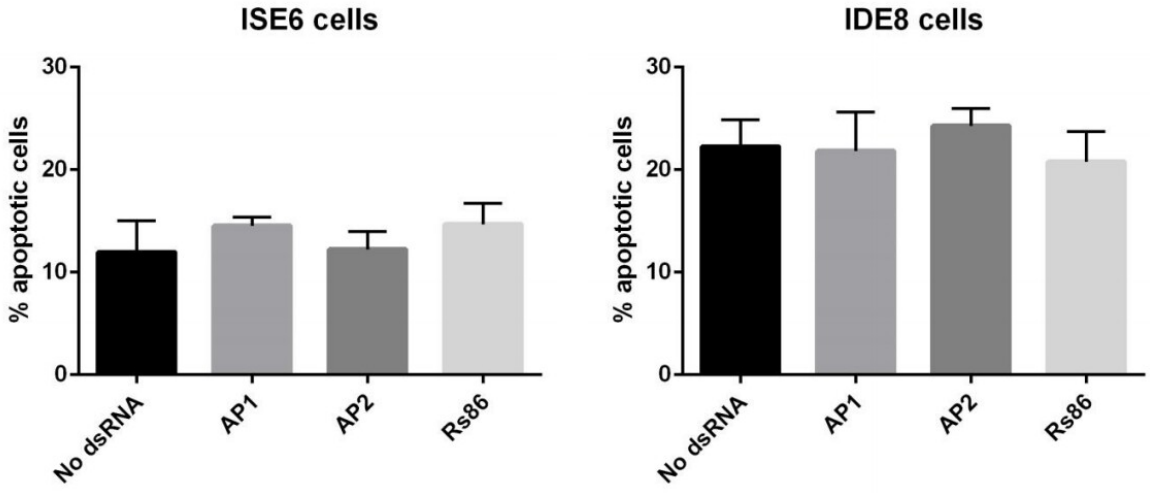

Figure 1 - Effect of target genes knockdown on cell viability of ISE6 and IDE8 tick cell lines. A and B. No significant differences were observed.

Bibliography:

[1] Alberdi, Pilar, et al. "Infection of Ixodes spp. tick cells with different Anaplasma phagocytophilum isolates induces the inhibition of apoptotic cell death." Ticks and tick-borne diseases 6.6 (2015): 758-767.

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