Protim

Download a copy here

Identification of new IRES-transactivating factors leading to the MELOE-1 translation using a combination of surface plasmon resonance and mass spectrometry approaches.

Maud Charpentier[1], Mike Maillasson[1-2], Agnès Fortun[1], Charles Pineau[3-4], Emmanuelle Com[3-4], François Lang[1]

[1] CRCNA, INSERM, CNRS, Université d'Angers, Université de Nantes, Nantes, France
[2] Impact, SFR santé F. bonamy, Inserm, CNRS, Université de Nantes, Nantes, France
[3] Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR_S 1085, F-35000 Rennes, France
[4] Protim, Univ Rennes, F-35000 Rennes, France

Introduction

MELOE-1 is a highly specific melanoma antigen involved in T cell immunosurveillance, which is translated from meloe, a long «non coding» and polycistronic RNA found in all cells of the melanocytic lineage (melanoma cells and normal melanocytes). The translation of MELOE-1 is however restricted to melanoma cells and is possible by the presence of an upstream IRES. IRES sequences are highly structured RNA sequences located upstream ORFs that allow the direct recruitment of ribosomal subunit and thus cap-independent translation of the ORF. Furthermore, translation initiation at eukaryotic IRES generally require the binding of proteic factors called ITAF in addition to the secondary RNA structure. ITAF implicated in MELOE-1 translation are not characterized yet and in this context we developed an ITAF identification method combining surface plasmon resonance and mass spectrometry approaches to identified new proteins interacting to the MELOE-1 upstream IRES sequence.

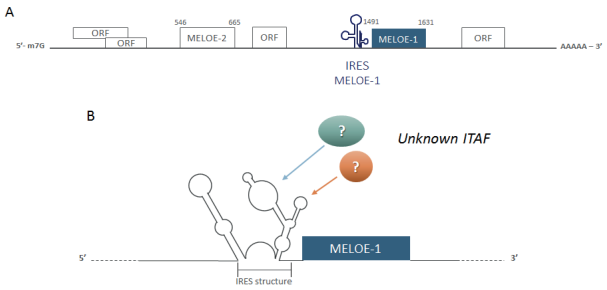

Figure 1: Schematic representation of the long «non coding» meloe RNA (A) with the structure of the IRES sequence of the MELOE1 antigen (B)

Methods

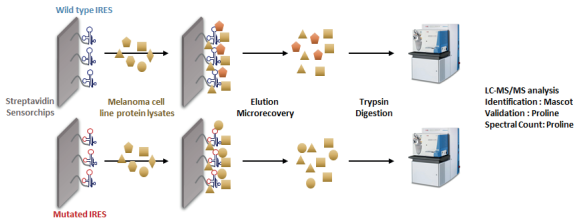

Figure 2: . Biotinylated target RNA sequences (wild type or mutated IRES without ITAF recognition sequence) were separately immobilized on biacore streptavidin sensorchips and their interacting proteins from melanoma cell line lysates were isolated with a Biacore instrument. After elution IRES interacting proteins were identified by nanoLC-MS/MS using a classical bottom-up database driven proteomics approach. The differential fixation of identified proteins on the two IRES sequences was evaluated by a spectral count approach.

Results

Some proteins from melanoma cell line lysates bind to the wild type IRES

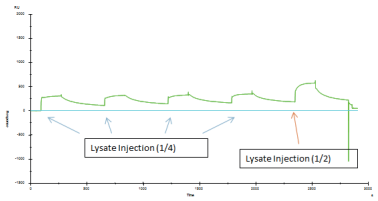

Figure 3: SPR sensograms showing the interaction of melanoma cell line lysates with the wild type biotinylated IRES which is immobilized on a streptavidin sensorchip.

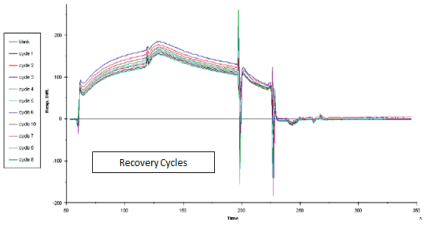

Figure 4: Proteins which bind to the IRES were recovered after several injection cycles for further identification by mass spectrometry.

Several RNA binding proteins were identified in the wild type IRES samples

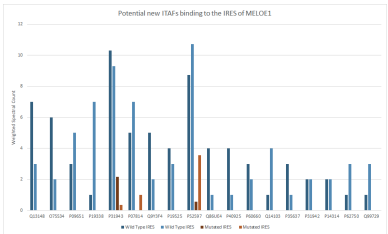

Figure 5: Spectral count quantification of several melanoma lysates proteins which interact specifically with the wild type IRES (beta-binomial p-value < 0.05).

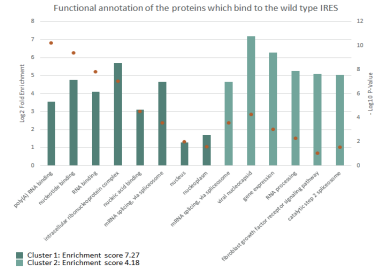

Figure 6: The enriched GO terms analysis of the proteins binding to the wild type IRES showed that they are involved in RNA binding, processing and splicing.(David Functional Annotation Clustering).

Conclusions & Perspectives

The combination of surface plasmon resonnance and mass spectrometry allowed the identification of new potential ITAF involved in the MELOE 1 translation.The binding of one protein of interest, previously described as a potential ITAF, to the MELOE 1 IRES has been confirmed by Biacore assay. Moreover, the IRES mutation of the potential interaction site with this protein decrease significantly the fonctionality of IRES in a bicistronic model of transfected melanoma cells. Our results showed that the combination of surface plasmon resonnance and mass spectrometry is useful to discover new RNA interacting partner.

Biogenouest

Protim web site :

References :

[1] Carbonnelle *and al*, 2013. PLoS One. 8 (9):e75233.

Abbreviations : IRES=Internal Ribosome Entry Site ; ITAF=IRES Transactivating Factor ; SPR: Surface Plasmon Resonnance

irset Inserm Université de Rennes 1 EHESP