SHORT VISIT GRANT_SCIENTIFIC REPORT
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ROLE OF THE FATE1 PROTEIN IN THE REGULATION OF MITOCHONDRIAL CALCIUM INFLUX

PURPOSE OF THE VISIT

Steroidogenic Factor-1, a nuclear receptor transcription factor, has a pivotal role in adrenal and gonadal development in humans and mice. We have defined a critical role of SF-1 dosage in regulating the proliferation of human adrenocortical cells and in the process of adrenocortical tumorigenesis in mice (Doghman *et al*., 2007). Our gene expression profiling study showed that an increased SF-1 dosage modulates the expression of transcripts involved in multiple functions and allowed us to identify FATE1 as a new target of SF-1.

FATE1 (X-linked Fetal and Adult Testis Expressed-1), which maps to chromosome Xq28, was identified as a cancer testis (CT) antigen-encoding gene expressed in hepatocellular carcinomas (Olesen C *et al.,* 2001 ; Dong XY *et al,* 2003). Some data suggest that FATE1 may represent an important factor in regulating the proliferative capacities of several types of cancer cells (Yang XA *et al.,* 2005).

In our cellular model of H295R/TR SF-1 cells overexpressing SF-1 in a doxycycline inducible fashion, we have shown that overexpression of SF-1 activates the expression of FATE1 at the transcript and protein level, through the presence of a binding site for SF-1 in the promoter of the gene. To date, the role of FATE1 is unknown.

We determined the subcellular localization of FATE1 using immunoelectron microscopy, and we showed that FATE1 is localized to mitochondria although it does not have a consensus mitochondrial localization signal. By using different biochemical approaches, we characterized the topology and orientation of FATE1 in the mitochondria and identified the domain involved in outer mitochondrial membrane anchorage.

By performing immunoprecipitations experiment followed by MS/MS analysis, we identify FATE1-interacting proteins, and among them, we found an Endoplasmic Reticulum (ER) interacting protein.

So the aim of my short visit in Prof. Rizzuto's Laboratory was to study the effect of FATE1 expression on calcium (Ca^{2+}) release and influx in ER and mitochondria.

Prof. Rizzuto introduced an innovative methodology for measuring Ca^{2+} concentrations in defined cell compartments, based on the molecular engineering and specific targeting of recombinant luminescent (aequorin) and fluorescent (GFP) proteins. This new approach allowed to obtain novel insight in the field of calcium signalling in mitochondria (Rizzuto R et al., 1992).

DESCRIPTION OF THE WORK CARRIED OUT DURING THE VISIT

To better understand the impact of FATE1 expression in calcium influx, we used two different cellular models.

First, we used H295R/TR SF-1 cells, overexpressing SF-1 in a doxycycline (DOX) dependent manner, leading to FATE1 expression. To study the specific impact of FATE1, we compared Ca^{2+} mitochondrial influx after cotransfection of cells with control or FATE1 siRNA and with a mitochondrial probe (Mitochondrial aequorin) in basal and DOX conditions.

The second cellular approach used to measure Ca^{2+} in cytosol and mitochondria were Hela cells cotransfected transiently with a FATE1 expression vector and its negative control with a mitochondrial or cytosolic aequorin probe. We tested also two other FATE1 constructions, the N-terminal part and the C-terminal part of FATE1 protein that lost or conserved mitochondrial localization, respectively.

After cotransfection with the appropriate aequorin probe, cells were reconstituted with coelenterazine and transferred to a perfusion chamber where light signal was collected by a luminometer and calibrated into calcium concentration values as previously described by Prof. Rizzuto (Rizzuto R et al., 1992).

DESCRIPTION OF THE MAIN RESULTS OBTAINED

During my short visit, I tested different methods of transfection in HeLa cells (calcium phosphate, lipofectamine) to measure the effect of FATE1 expression in mitochondrial and cytosolic calcium concentrations.
Unfortunately, we experienced problems with calcium phosphate transfection, while lipofectamine transfection seems to affect the level of cellular calcium measurement. However, the preliminary results seem to indicate an effect of FATE1 in mitochondrial calcium influx. Further experiments are necessary to confirm these results.
Concerning our inducible human adrenocortical cellular model, H295R/TR SF-1 no data exist in the literature that used this methodology for calcium measurement in those cells. So I started to optimize the condition of cotransfection of mitochondrial and cytosolic aequorin with FATE1 siRNA, and also to test different stimuli for the optimal calcium response in H295R/TR SF-1 cells. The first results were encouraging but further optimizations are necessary to clarify the impact of FATE1 expression and silencing in calcium response.

FUTURE COLLABORATION WITH THE HOST INSTITUTION.

It seems evident that this first visit gave us the opportunity to establish a new and strong collaboration with Prof. Rizzuto Laboratory.
Nevertheless, many optimizations in cells transfection methods are necessary to confirm our preliminary results, particularly in H295R cells.
On my return in lab, I will try different methods of transfection to obtain the highest efficiency for both the aequorin probe and FATE1 siRNA.
After this optimization step, I will schedule another visit in Prof. Rizzuto's Laboratory for calcium measurements.

Another interesting point is the potential interaction of FATE1 with ER protein components. Many cellular processes require the cooperation between mitochondria and endoplasmic reticulum. Several recent works show that their functional interactions by structural contacts, called MAMs (Mitochondrial associated membrane) are crucial in metabolism, cell survival and cell death as well as intracellular calcium signaling (Pinton P et al, 2007; De Brito OM and Scorrano L, 2010).
Thus, I will use both biochemical and immunofluorescence approaches to observe if FATE1 is localized in those MAMs structures.

PROJECTED PUBLICATIONS.

We showed that FATE1 is localized to mitochondria. We characterized the topology and orientation of FATE1 in the mitochondria and identified its domain involved in anchoring to the outer mitochondrial membrane.
Moreover, in collaboration with Dr Fassnacht's group (Würzburg, Germany) we observed a strong FATE1 staining signal in human ACC compared to normal adrenal gland. Interestingly, it appears that there is a strong correlation between SF-1 and FATE1 expression and overall or recurrence-free survival in patients. FATE1 appears to be a potential marker in human adrenocortical tumors. So these studies on FATE1 cellular function are necessary to understand its role in adrenocortical tumorigenesis. These important results will be submitted for publication as soon as possible.

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