Diagnostic des maladies génétiques par l'analyse de la signalisation calcique et des cellules foetale circulantes

Rapport Hcéres

agence d'évaluation de la recherche
et de l'enseignement supérieur

Section des Unités de recherche

Evaluation report

Research unit :

Diagnostic des maladies génétiques par l'analyse de la signalisation calcique et des cellules fœtales circulantes

of the University Paris 5

Mars 2009

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of the University Paris 5

Le Président de l'AERES	Section des unités de recherche
	Le Directeur
Jean-François Dhainaut	**Pierre Glorieux**

mars 2009

Evaluation report

The research unit :

Name of the research unit : Diagnostic des maladies génétiques par l'analyse de la signalisation calcique et des cellules foetales circulantes

Requested label : UMR_S INSERM

N° in case of renewal : 807

Head of the research unit : Ms. Patrizia PATERLINI

University or school :

Université Paris 5

Other institutions and research organization:

INSERM

Date of the visit :

January 30th 2009

Members of the visiting committee

Chairman of the commitee :

M. Joel NARGEOT, University of Montpellier 2

Other committee members : Paolo Bianco (Roma)

M. Philippe LE BOUTEILLER, University of Toulouse 3

M. Philippe GAILLY, Catholic university of Louvain, Belgium

CNU, CoNRS, CSS INSERM, représentant INRA, INRIA, IRD.....) representatives :

M. Hervé PRATS, INSERM

Observers

AERES scientific representative :

M. Thierry RABILLOUD

University or school representative :

M. Arnaud DUCRUIX, Université Paris 5

Research organization representative :

Ms. Chantal LASSERRE, INSERM

Evaluation report

1 • Short presentation of the research unit

Total number of lab members: 12

- Number of researchers with teaching duties : 1
- Number of full time researchers : 0
- Number of postdoctoral fellows : 3
- Number of PhD students :4, all with funding
- Number of engineers, technicians and administrative assistants : 4
- Number of HDR : 1
- Number of students who have obtained their PhD during the past 4 years : 2
- Average length of a PhD during the past 4 years : 4 years
- Number of "publishing" lab members: 1 out of 1

2 • Preparation and execution of the visit

The visit was well prepared. All members of the review panel got before the site visit the necessary scientific and administrative documents for a proper evaluation of the scientific activity of the Unit and its project.

The site visit was well organized, and enough time was allocated for all subheadings of the visit. After the scientific presentations made by the director of the research unit, then by the researchers in charge of each subproject, i.e. the principal investigator as well as the postdoctoral fellows, the expert review panel could meet the representative of Paris 5 University and IFR. The committe split to meet the different staff categories: Postdocral fellows+ PhD students, technicians and administrators. A visit of the laboratory was then made to end the auditing process. The committee deliberated and reached a consensus view of the laboratory.

3 •Overall appreciation of the activity of the research unit, of its links with local, national and international partners

The group, despite its small size, has been competitive in the fundamental research area concerning the role of calcium signaling in pathologies related to ER stress and mitochondria dysfunction. In particular they focused on the novel concepts of the communication between intracellular organelles and highlighted the role of a truncated isoform of the SERCA pump which might be considered as a ER-stress protein and responsible for Ca++ leak from ER to mitochondria. Ca++ overload in mitochondria mediate apoptosis and cell death. The second point is the development of a non invasive diagnosis test (ISET) for the detection of circulating fetal cells with potential clinical applications in several common genetic diseases. The members of the unit have convincingly demonstrated the value of its use for prenatal diagnosis of SMA and cystic fibrosis and plan to investigate its efficiency in trisomy 21. Patents have been obtained belonging to Inserm/university/AP/HP.

4 • Specific appreciation team by team and/or project by project

Calcium homeostasis plays a pivotal role in many cellular functions. Ca signals result first from Ca influx through opening of plasma membrane calcium channels. Ca release from intracellular stores (SR and ER) through Ryanodine or IP3 receptors. Their amplitude and duration is dependent on both the kinetics of opening and closing of these calcium channels proteins but also from removal of intracellular calcium by various pumps such as membrane Ca ATPases on the plasma membrane (PMCA) or on the sarco-endoplasmic membranes (SERCA) and transporters (such as the Na/Ca exchanger). These various proteins allow to produce calcium signals which are specific in space, time and amplitude and to control specific cellular responses in various tissues. While the role of the calcium channels, pumps and transporters is rather well established in terms of the dynamics of the calcium signals, the role of mitochondria remains more obscure and in particular Ca++ transport between intracellular organelles and mitochondria. The project of the unit has been based on the crosstalk between these organelles with the hypothesis of close contacts existing between ER and mitochondria playing a major role in pathologies such as mitochondrial Chain respiratory diseases (MRCD) or in ER stress.

The unit has focused a complex II mutation of MRCD related to a pathology (Leigh's symdrome) by developing a transversal study involving the isolation of fibroblasts from patients harbouring this mutation. Thanks to the collaborations with an excellent group of the university of Ferrara which allow them to use cytosolic and targeted mitochondrial and ER fluorescent probes introduced through an adenoviral strategy. This approach allows assessing the calcium concentration in the various compartments and organelles in response to agonist evoked responses. The results demonstrated mitochondrial Ca++ overload, increase ER Ca++ leak and increased basal and stimulated cytosolic Ca++ levels. This study emphasizes the links between Ca++ signalling through the different intracellular compartments, the production of ATP and ROS and mitochondrial potential. A publication for J. Biol. Chem. is in favourable revision.

The second aspect concerns the ER stress in relation with inter-organelle Ca signalling. In ER-stress conditions, the mitochondrial Ca++ overload drives apoptosis and the mechanisms involved in ER-mitochondria Ca++ transfer are postulated to be of major importance. Their studies highlight the role of a SERCA1 truncated isoform (S1T) in the control of the ER-stress response and cell death. S1T overexpression was observed in several pathophysiological ER-stress models and the mechanism of the S1T and the group further investigated S1T induction. S1T induction by ER-stress is induced by the ATF4 pathway involving an interaction with CHOP transcription factors and resulting in ER Ca++ leak. Using S1T RNAi transfection, they were able to show reduced steady state ER Ca and increased ER Ca leak. S1T is considered as an ER-stress protein. Various approaches including western Blots, high resolution imaging evidenced that S1T co-localizes with mitochondria and could mediate SR leak during ER-stress in the ER-mitochondria microdomains. These quite interesting and novel data were published in the excellent journal Mol Cell in 2008 .

The project is based on these results which have evidenced the crosstalk between mitochondria and Endoplasmic reticulum and the role of S1T overexpression in different ER stress models. It proposes to investigate these molecular mechanisms in another ER-stress human pathology: the Alzheimer disease. Mitochondrial dysfunction is reported in AD and is able to trigger neuronal apoptosis. It is postulated in this project that the toxicity of amyloid species might involve the calcium signalling crosstalk between the organelles. Several pieces of evidence lead to this hypotheses, such as specific accumulation of APP in mitochondria of diseased subjects which is correlated with the severity of the disease. The aims of the study are first: to evaluate mitochondrial and ER intercommunication in AD models. This includes studies of mitochondria structure and dynamics and looking for evidences for contact sites. Second, to understand the molecular mechanisms underlying the ER-stress mediated neuronal apoptosis. Third: investigate the role of the truncated isoform S1T of the SERCA1 gene in AD. This aspect is very novel and strong arguments including the overexpression of S1T in AD brains are supporting the hypothesis. The project received recently substantial funding by the FRM (for 3 years) which further attests the quality of the project, such granting being very competitive.

The group has developed a method for the isolation by size of circulating non-hematopoietic (epithelial, trophoblastic) fetal cells (ISET, Lancet 2003), and has been using this approach as a potential tool for the non-invasive prenatal diagnosis of monogenic diseases. In principle, the same approach could have significant value in other areas, such as oncology. Although this is recognized by the group under review, and duly mentioned in the report material provided, activity seems to have focused on the prenatal diagnosis application.

Activities in this areas have been marked by a strong translational commitment with the development of two patents and the creation of a company (Metagenex) for the further development and exploitation of protected intellectual property. This seems to have happened in response to a national law and political drive towards the translation of fundamental research to transferable technology. It appears that accidents have prevented the expected or planned development of the creation of the company. While this would have bearing on overall considerations and future planning, it is felt that appraisal of these circumstances clearly exceeds the scope of a scientific review. Three major papers produced in this area are marked as submitted at the time of this review. Data generated in this area relate to 4 different topics:

- Validation of the approach for one monogenic disease (spinal muscular atrophy)
- Definition of the timing of appearance and persistence of circulating trophoblastic cells in the maternal bloodstream
- Development of an ISET based approach to the non-invasive diagnosis of cystic fibrosis
- Analysis of CFTC in the blood stream of women receiving implantation of multiple embryos after in vitro fertilization

5 • Appreciation of resources and of the life of the research unit

The ressources of the unit come from different sources : INSERM annual support around 70 to 80 K€ and grants from various caritative associations such as ARC, VLM, AFM. The group belongs to an european network of excellence (SAFE). This approximatively doubles the amount of granting by the institution. A substantial FRM grant (350 K€ for 3 years) has been obtained for the project and private grants are expected for the validation of ISET technology for the diagnosis of several genetic pathologies. Overall, the budget is considered as relevant to the projects. The committee has interviewed the members of the unit (including post doc, PhD students, technicians, admistratives) ans has appreciated the excellent relationships between all the members of the unit and their strong motivation for the project and their clear support to the director of the unit.

6 • Recommendations and advice

– **Strong points :**

A strong point is the quality of the research in respect to the small size of the group composed of only one permanent researcher (the director), post docs and PhD students. They have attained a very good level of publications, have been successful in finding significant amounts of funds from various sources including European, have established several strong foreign collaborations. The Director has gained international visibility (many invitations to meetings) and introduced novel concepts in calcium signalling, showing the importance of the communication between intracellular organelles during ER stress, in particular the role of a truncated SERCA isoform. The project proposes, based on these recent findings, to study the calcium signalling crosstalk between intracellular organelles in Alzheimer disease (also considered as a ER-stress pathology). The role of the S1T isoform in the development of the disease will be examined. The preliminary data support at least this hypothesis of modifications of the expression of S1T in AD models.

The committee was impressed by the motivation and the friendly attitude between all members of the group as well as their willingness to pursue this new project with the director.

The team is well integrated on the site and has open access to the different facilities (platforms) of the IFR and INSERM center.

Considering the second research axis, the technical approach that forms the basis of this line of activity is simple and ingenuous and per se seems to carry significant applicative potential. The areas of potential application of the approach have been correctly and timely identified and pursued. A necessary “translational” attention to the development of protected IP has characterized the activity of the unit in this area.

Data on the validation of the technique for the non-invasive prenatal diagnosis of SMA as compared to chorionic villous sampling are convincing and just impressive. Data on the detection of CFTC with multiple genotypes in mothers

receiving the implantation of multiple IVF-generated embryos are also significant, and do provide novel information of general significance and further scientific and applicative potential.

- **What needs to be improved :**

What might appear as a weak point is the small size of the group with two different research directions. However, as mentioned before, the group participates to the scientific life of the site and has open access to a number of technical facilities of the site. To maintain some continuity in the research programs and keep the team specific skills, it seems needed to add a permanent young researcher in the fundamental research team. One potential candidate already on site has been identified. One talented post doc is leaving the group for joining another research institute in Italy, but intends to keep tight scientific relashionships with the team and plans to come back and join the team as soon as possible. Considering the recent publication list and submitted papers, one can expect a young fellow to get an institutional position (CNRS or Inserm) or more probably to join the university with a position of "Maître de Conférences". Appropriate "lobbying" should be undertaken.

It will be useful to extend the evidence for the specificity and sensitivity of the ISET technique in other diseases. As reproducibility, consistency and reliability of the sampling per se, rather than use of the detected cells for diagnosis, seem the major remaining rate-limiting steps with this technique as a general approach, it will be important to gain a more extensive understanding on the technical possibility to detect CFTC in larger cohorts of women, and on aspects of timing. Technical alternatives to microdissection (e.g., sorting of single cells) could also be explored.

- **Recommendations :**

Following the previous comments, the committee recommends to the director to act for increasing the critical mass of the group by recruiting at least one young permanent researcher. We noticed that all the PhD students present in this laboratory should finish their thesis within a year or less. Thus there is a clear need of new PhD students.

We also recommend to the director to join a research center in the future, to further increase the stability and visibility of the group. The director has already indicated that she is involved and wishes to join the project "IMAGENE" which is associated with the construction of a new building.

Concerning the clinical research, development of this original approach seems warranted, and it is to be encouraged. It appears that this activity could represent a direct applicative output for a group otherwise committed to more fundamental research of high quality. In planning the growth and composition of the unit, it will be important to keep in mind the distinction of the two lines of activity, which obviously cannot be conducted with the required impact unless people involved are dedicated and focused.

Diagnostic des maladies génétiques par l'analyse de la signalisation calcique et des cellules

Note de l'unité	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	A	A	A

UNIVERSITÉ
PARIS DESCARTES

Le Président
Axel KAHN

Paris, le 3 avril 2009

DRED 09/n° 135

Monsieur Pierre GLORIEUX
Directeur de la section des unités de l'AERES
20 rue Vivienne
75002 PARIS

Monsieur le Directeur,

Je vous remercie pour l'envoi du rapport de comité de visite concernant l'unité « **UMR-S 807 Diagnostic des maladies génétiques par l'analyse de la signalisation calcique et des cellules fœtales circulantes** » rattachée à mon établissement.

L'Université a pris bonne note des remarques du comité de visite et veillera, en partenariat avec l'INSERM, à ce que les recommandations faites soient suivies d'effet.

Je vous prie de croire, Monsieur le Directeur, à l'expression de ma meilleure considération.

Le Président de l'Université

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Paris, April 1st 2009

To whom it may concern

AERES report of Unit 807

Document about observations

We thank the visiting committee Chairman, AERES scientific representative and all the Committee members for the enriching work and deep analysis of our research project they have done.

About the Committee recommendations:

- we take into deep consideration the Committee's encouragement to increase the size of our Unit. We have already taken action in this direction (meeting with the Dean, contacts etc). We also have accelerated the publication rhythm in order to provide the best chance to the post-doc on site who will postulate for a Maitre de Conférence position. The talented post-doc who works in the Research Center in Genova will apply for a permanent INSERM position this year. We thus hope to reinforce our fundamental research team.
- We have accepted a PhD student (co-tutoring Italy-France) for the next year and will accept another Master 2 and another PhD student for next year.
- Our Unit also aims to join a Research Center in the future. It is involved in the IMAGINE project and acts actively to be selected in the new IMAGINE building. In parallel, it also keeps close contacts (common meetings etc) with the Research Center in Necker.
- Concerning the ISET projects, we have already established a collaboration with Professors Brice and Dommergues to develop the non invasive prenatal diagnosis of Huntington disease. We will proceed through technical validation (sensitivity, specificity of the molecular method) followed by clinical validation. We also aim and act to find funding allowing to validate the items of number of circulating trophoblastic cells and timing of circulation

in large cohorts of pregnant women. Dr Pfeifer (senior post-doc, CDD INSERM) is in charge of the molecular and technical aspects of the ISET method (alternatives to microdissection, Trisomy 21 project etc...) and will apply for an INSERM permanent position in 2 or 3 years. We also look for establishing or reinforcing the collaboration with an academic obstetrician who will refer to our INSERM Unit and will be in charge of the clinical trials.

Prof. Patrizia PATERLINI-BRECHOT
Director U.807 INSERM