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CROSS-BORDER ITALY-SLOVENIA BIOMEDICAL RESEARCH:

ARE WE READY FOR HORIZON 2020?

The Cross-Border Cooperation Programme Italy-Slovenia 2007-2013 has funded 6 projects targeting biomedical research. For the first time, researchers from 44 project partners have met to share their results and future perspectives.

CONFERENCE PROCEEDINGS

WITH AN ANALYSIS OF INNOVATION MANAGEMENT AND KNOWLEDGE TRANSFER POTENTIAL FOR A SMART SPECIALIZATION STRATEGY

ATTI DELLA CONFERENZA - ZBORNIK

2007-2013
cooperazione territoriale europea
programma per la cooperazione transfrontaliera
Italia-Slovenia
evropsko teritorialno sodelovanje
program čezmejnega sodelovanja
Slovenija-Italija

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TRIESTE 2014

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FACT SHEET

The six biomedical research projects of the Cross-Border Cooperation Programme Italy-Slovenia 2007-2013

PROJECT	CALL	DURATION	LEAD PARTNER & COORDINATOR	N OF PARTNERS	BUDGET (€)
TRANS2CARE	01/2009	Apr 2011 Sep 2014	Università degli Studi di Trieste *Sabina Passamonti* www.trans2care.eu	13	*2.611.118*
PANGEA	02/2009	Oct 2011 Sep 2014	ZRS – Znanstveno raziskovalno središče - Univerza na Primorskem *Rado Pišot* www.pangeaeu.org	9	*1.253.752*
GLIOMA	02/2009	Nov 2011 Oct 2014	Morska biološka postaja Nacionalni inštitut za biologijo *Tamara Lah Turnšek* www.glioma.eu	5	*1.320.000*
SIGN	02/2009	Nov 2011 Oct 2014	Univerzitetni klinični center Ljubljana *Borut Peterlin* www.signgenetics.eu	8	*1.285.441*
MINA	03/2011	Oct 2012 Mar 2015	SISSA - Scuola Internazionale Superiore di Studi Avanzati *Stefano Gustincich* www.minaproject.eu	4	*998.293*
PROTEO	03/2011	Oct 2012 Apr 2015	Elettra - Sincrotrone Trieste S.C.p.A. *Paola Storici* www.elettra.eu/Prj/PROTEO/	5	*992.771*
			TOT	**44**	***8.461.315***

Il volume raccoglie gli atti della Conferenza Biomedica Transfrontaliera tenutasi all'Università di Trieste il 27 febbraio 2014.

L'opera è redatta in tre lingue: italiano, sloveno e inglese, avendo come scopo principale raggiungere una platea multiculturale e multidisciplinare più vasta possibile; fanno eccezione alcuni contributi a carattere prettamente specialistico.

Tre sezioni distinte seguono l'iniziale presentazione degli obiettivi e delle caratteristiche salienti della Conferenza.

La prima sezione raccoglie la descrizione dei cinque progetti biomedici finanziati dal Programma per la Cooperazione transfrontaliera Italia-Slovenia 2007-2013 – Trans2Care, Glioma, Mina, Pangea, Proteo e Sign, per mano dei rispettivi Coordinatori.

La seconda raccoglie i contributi tecnico-scientifici, a testimonianza dell'ampiezza e carattere d'avanguardia delle competenze presenti nell'Area transfrontaliera.

La terza parte raccoglie alcuni articoli che elaborano e approfondiscono i temi politici affrontati nella Conferenza.

La vivace discussione cha ha caratterizzato la Conferenza ha riguardato la sentita necessità di unire le forze per migliorare la qualità delle ricerche, da una parte, e l'efficienza nell'acquisizione di fondi europei, dall'altra. È fortemente sentita pure l'esigenza che i decisori politici siano maggiormente consapevoli dell'enorme patrimonio di competenze umane, esperienza storica e infrastrutture materiali d'avanguardia presenti nell'Area Programma. Un patrimonio che dovrebbe essere sfruttato a fondo per contribuire in modo significativo alla crescita intelligente, sostenibile e solidale di queste Regioni.

Pričujoči zbornik Čezmejne biomedicinske konference, ki je potekala 27. februarja 2014 na Univerzi v Trstu, vsebuje prispevke udeležencev konference.

Knjiga je napisana v treh jezikih: italijanščini, slovenščini in angleščini. Cilj knjige je doseči ljudi iz različnih kultur in/ali strokovnega predznanja.

Izjema so le določeni prispevki, ki imajo posebne tehnične in znanstvene vloge.

Po uvodni predstavitvi namena konference sledijo trije različni tematski sklopi.

V I. delu so zbrani opisi petih biomedicinskih projektov, ki jih financira Program čezmejnega sodelovanja Slovenija-Italija 2007-2013, in sicer Trans2Care, Glioma, Mina, Pangea, Proteo in Sign. Prispevke so napisali vodje posameznih projektnih ekip.

V II. delu so dokumenti o tehničnih in znanstvenih temah, ki predstavljajo širino in znanstveno odličnost, ki je na voljo v Slovenija-Italija čezmejnem območju sodelovanja.

V III. delu je zbirka člankov, ki se nanaša na politična vprašanja, katerih se je vsebinsko dotaknila konferenca.

Ob zaključku konference je sledila intenzivna razprava o združitvi moči, da bi izboljšali kakovost raziskovalnega dela in stopnjo uspešnosti pri pridobivanju evropskih sredstev.

Udeleženci konference prav tako upajo, da se bodo politični odločevalci pričeli zavedati ogromne zaloge človeškega kapitala in raziskovalne infrastrukture, ki je na voljo na programskem območju. To je potrebno ustrezno izkoristiti za pametno, trajnostno in vključujočo rast teh regij.

The current book Proceedings of the Cross-Border Biomedical Conference, held at the University of Trieste on 27th February 2014, collects the contributions from the conference participants.

The document is presented in three languages: Italian, Slovene and English. Its aim is to reach people from a wide range of cultures and/or backgrounds of expertise.

The only exceptions are some contributions which have specific technical and scientific elements.

Three distinct sections follow the initial presentation of the objectives and features of the Conference.

Part I collates the description of the five biomedical projects funded by the Cross-border Cooperation Programme Italy-Slovenia 2007-2013, i.e. Trans2Care, Glioma, Mina, Pangea, Proteo and Sign. The respective team managers wrote the contributions.

Part II collates the papers on technical and scientific topics, which give an idea of the breadth and cutting-edge character of the expertise available in the Italy-Slovenia cross-border cooperation area.

Part III is a collection of papers that elaborate on the political issues addressed by the Conference.

An intense discussion at the end of the Conference addressed the urgent need to unite efforts to improve both the quality of our scientific work and to increase our capacity to attract European funds. The participants also expressed the hope that political decision makers become aware of the immense stock of human capital and research infrastructure available within the Programme Area. This should be properly exploited for a significant contribution to the smart, sustainable and inclusive growth of these Regions.

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SEARCH FOR COMPOUNDS ABLE TO MODULATE F_0F_1 ATP SYNTHASE IN SWITCHING FROM LIFE ENZYME TO CELL DEATH EXECUTOR

Marco Zancani[1], Giovanna Lippe[2], Enrico Braidot[1], Elisa Petrussa[1], Valentino Casolo[1], Carlo Peresson[1], Sonia Patui[1], Alberto Bertolini[1], Valentina De Col[1], Paolo Bernardi[3], Angelo Vianello[1]

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[3] University of Padova, Dept. of Biomedical Sciences, Padova

Abstract — Mitochondria are the main site of energy power in eukaryotic cells. The enzyme F_0F_1 synthase is responsible for ATP production driven by the transmembrane proton gradient. The maintenance of a very low permeability of the inner mitochondrial membrane is crucial for this mechanism, since sudden opening of the permeability transition pore (PTP) leads to matrix swelling and outer membrane rupture, with release of pro-apoptotic factors. Recently, it has been suggested that dimers of ATP synthase in mammals could represent the main component of the mitochondrial PTP, a feature modulated by calcium and involving the matrix protein Cyclophilin D (CyPD). This study would help to develop new tools for the identification of plant secondary metabolites, in particular flavonoids, able to modulate PTP and therefore acting on the programmed cell death mediated by mitochondria. Therefore, this project would represent the first screening for plant molecules able to interfere with programmed cell death, as a preliminary study for the development of drugs active in PTP-related pathologies.

Index Terms — TRANS2CARE, Cyclophilin ATP synthase, permeability transition pore, programmed cell death, cyclophilin D

1 BACKGROUND

Mitochondria are double-membrane organelles, which represent the energy power of eukaryotic cells. In aerobic conditions, the production of ATP in mitochondria is exerted by F_OF_1 ATP synthase. Nevertheless, mitochondria are also involved in the early stages of programmed cell death (PCD) through the release of pro-apoptotic factors from the intermembrane space. Such a release can be due to a sudden opening of the inner mitochondrial membrane permeability transition pore (PTP) to small molecules. This implies a matrix swelling, leading to outer membrane rupture and release of pro-apoptotic factors. Recently, it has been proposed that ATP synthase, when present as a dimer, forms the mitochondrial PTP in the presence of high calcium concentration (Bernardi, 2013). Consistently, the modulator of permeability transition, the matrix protein Cyclophilin D (CyPD), has been identified also as ATP synthase interaction factor (Giorgio et al., 2009).
Evidence of PTP formation by ATP synthase dimers has been obtained in mammalian mitochondria (Giorgio et al., 2013), although the underlying mechanisms remain to be established. Furthermore, little information is available for PTP in other organisms, where mitochondria are involved in PCD, in particular in plants (Vianello et al., 2012).

2 OBJECTIVES

This research will contribute to clarify the enzymatic mechanisms that allow the switch of ATP synthase to PTP formation and the physiological effectors involved in this process. Potential compounds, such as plant secondary metabolites (e.g. flavonoids), which could interfere with PTP formation will be identified. Then, their ability to modulate PCD pathway, mediated by mitochondria, will be examined. This would represent a preliminary step for the development of drugs active in PTP-related pathologies (tumors, ischemia, neurodegenerative diseases).

3 APPROACH & METHODS

General approach

The structural and functional properties of ATP synthase from different origins will be compared to define the essential elements involved in the process. This will constitute the basis for the following step in which molecules, able to modulate the PTP/ATP synthase switch activity, will be identified.

Methods

The structural and functional characterization of the ATP synthase will be performed by proteomic and immunological approaches. The mammal model will be used for comparison with ATP synthase from plant mitochondria. PTP formation will be followed by spectrophotometric and fluorimetric assays. The role of essential components of ATP synthase for PTP formation will be tested by knock-out mutants of Arabidopsis. The potential modulators of ATP synthase switch to PTP in both mammalian and plant mitochondria will be tested by fluorimetric techniques.

4 RESULTS

Dimerization of ATP synthase has been characterized in mammalian mitochondria by biochemical approaches, such as native electrophoresis, immunoblotting and enzymatic activity detected *in situ* (Giorgio et al., 2009). Identification of ATP synthase interaction factors has been performed by immunoprecipitation and cross-linking agents. It has been demonstrated that ATP synthase dimers

interact through the lateral stalk (OSCP subunit) with the matrix protein Cyclophilin D (CyPD), which is a well-known inducer of PTP closure, and such interaction is weakened by PTP-blocker Cyclosporin A (CsA) (Fig. 1) (Giorgio et al., 2009; 2013). Moreover, when isolated ATP synthase dimers were inserted into planar lipid bilayer, electrophysiological measurements demonstrated that, in the presence of calcium, they are able to form a channel with properties identical to those described for PTP (Giorgio et al., 2013). A hypothetical model of ATP synthase transition to PTP has been then formulated (Bernardi, 2013) (Fig. 2).

Plant mitochondria possess a similar active ATP synthase complex, but it has not been associated to PTP formation yet. Nevertheless, some components have been identified, such as OSCP and CyPD, but it is still not clear if they could interact in PTP modulation.

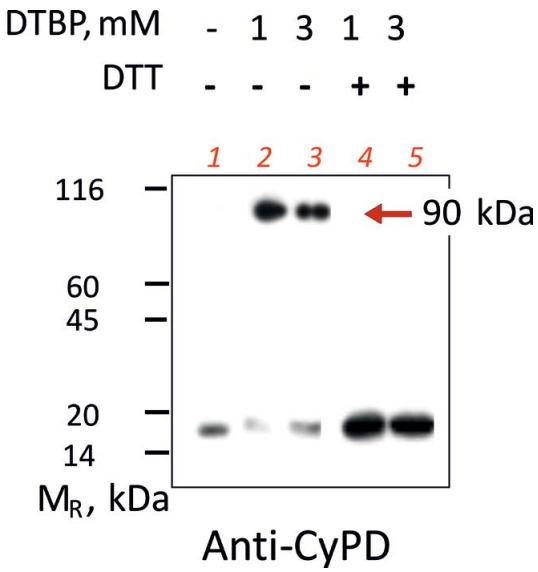

Figure 1: CyPD interacts with mitochondrial ATP synthase through the lateral stalk connecting F_OF_1. ATP synthase from mammalian mitochondria was separated by SDS-polyacrylamide gel electrophoresis and immunodetected with Ab against CyPD, before (lane 1) and after treatment with the crosslinker DTBP in absence (lanes 2 and 3) or presence of DTT (lanes 4 and 5) able to revert the effect. The 90 kDa crosslinked proteins (arrow) reacted also with Ab against the lateral stalk subunits of ATP synthase (not shown) (modified from Giorgio et al., 2009).

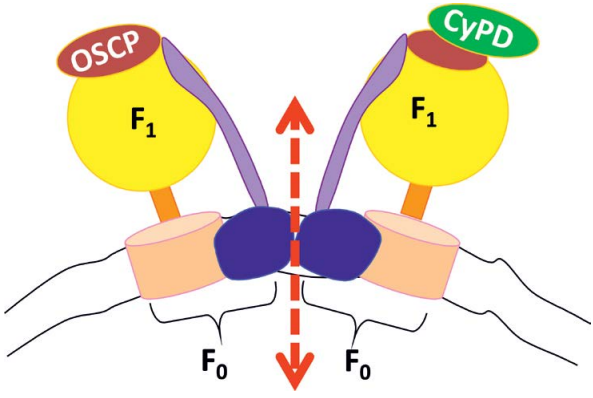

Figure 2: Hypothetical transition of F_OF_1 ATP synthase dimers to form the PTP. ATP synthase dimers can undergo PTP formation when Ca^{2+} rather than Mg^{2+} is bound, possibly at the catalytic sites, in a reversible process favored by binding of CyPD to OSCP. Adenine nucleotides counteract PTP formation in synergy with Mg^{2+}. Red arrow denotes the hypothetical pathway for solute diffusion between two FO subunits (adapted from Bernardi, 2013).

5 POTENTIAL NEW PRODUCTS & SERVICES

This study will allow the identification of plant secondary metabolites (i.e. flavonoids) able to modulate ATP synthase switch to PTP. This would represent the first screening toward the realization of potential natural pharmaceuticals/drugs able to interfere with programmed cell death. Furthermore, the analysis of the components involved in ATP synthase activity and regulation would lead to identify proteins correlated to PCD. Their quantification in different organisms/tissues by immunological/proteomic techniques would lead to design diagnostic tools for PTP-related pathologies.

6 CURRENT COLLABORATIONS

6.1 With other researchers

University of Padova, Department of Biology, Department of Biomedical Sciences; University of Udine, Department of Food Sciences; University of Trieste, Department of Life Sciences (LP, Trans2Care).

7 CONTACT OR COLLABORATIONS NEEDED

Future collaboration with clinical laboratories is needed.

8 COMMUNICATION TOOLS

- The high level of expertise in the field of biochemistry, as well as the high level of applicability, quality and performance of the presented research will be disseminated through high quality scientific publications.
- This research has been presented to the biomedical community at National and International congresses.

9 FUNDS NEEDED

9.1 For basic research (investigation of biological mechanisms): € 40,000

9.2 For applied research (solutions for real-world problems): € 100,000

9.3 For pilot & demonstrator activities (to develop a prototype): € 150,000

10 CONCLUSIONS

The mitochondrial permeability transition is an essential event in mediating the early stage of the PCD pathways. The recent evidence of ATP synthase involvement in PTP formation opened an innovative and still unexplored research field to find agents able to modulate the PTP. This investigation would open new opportunities to identify plant natural products able to interfere with the PTP phenomenon and therefore would represent the first step to design diagnostic tools for PTP-related pathologies, which comprise a wide range of human diseases.

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