Planul Național pentru Cercetare-Dezvoltare și Inovare II

Denumirea proiectului: **„Monitorizarea statusului functional al celulelor electropermeabilizate in strategiile electrochimioterapeutice”**
Contract nr.: **30 / 09.06.2008, AAd. Nr. I/01.10.2009**
Durata contractului: **17 luni si 20 zile**
Valoarea contractului: **107477 lei**

Title: Monitoring the functional status of electropermeabilized cells in electrochemotherapeutic strategies
Contract no.: **30 / 09.06.2008, AAd. Nr. I/10.09.2009**
Duration: **17 months and 20 days**
Funds: **107477 lei**

Finanțare: **Buget de Stat -Autoritatea Nationala pentru Cercetare Stiintifica**
*Programul : **CAPACITATI***
Categoria de proiect: **modulul III**

Funds: **State Budget – National Authority for Scientific Research**
*Program: **CAPACITATI***
Project Category: **modulus III**

Partener roman
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Acces la facilitatile laboratorului

Laboratorul de Biofizica si Biotehnologie Celulara este deschis pentru studentii master si doctoranzi precum si pentru partenerii din proiectele de cercetare in zilele de Luni-Vineri, orele 8-20.

Program for research vistors (Master and Ph.D.students, research parteners)
Monday –Friday, 8-20

Lista echipei proiectului-partener roman
Prof. Dr. Kovacs Eugenia - director de proiect
Conf. Dr. Savopol Tudor - specialist
Sef lucr. Dr. Moisescu G.Mihaela – specialist
Dr. Stanciu Liana – specialist
Fiz. Surleac Marius – specialist
Ing. chim. Bajenaru Laura - specialist
Drd. Paraico Iurie – tanar cercetator
Fiz. Iordache Minodora – tanar cercetator
Biochim. Radacina Lavinia – tanar cercetator

Project team - romanian partner
Prof. Kovacs Eugenia (biophysicist)– romanian project coordinator
Assoc. Prof. Dr. Savopol Tudor (chemist) – researcher
Lecturer Moisescu Mihaela (medical doctor) - researcher
Stanciu Liana (medical doctor)- researcher
Surleac Marius (physicist) - researcher
Bajenaru Laura (chemist) - researcher
Paraico Iurie (medical doctor)– Ph.D. Student, young researcher
Iordache Minodora (physicist)– Ph.D. Student, young researcher
Radacina Lavinia (biochemist) – young researcher

Lista echipei proiectului-partener sloven
Prof. Dr. Miklavcic Damijan - director de proiect
Prof. Dr. Sersa Gregor - specialist
Dr. Pavlin Mojca – specialist
Dr. Kanduser Masa – specialist
Dr. Pucihar Gorazd – specialist
Drd. Haberl Sasa – tanar cercetator
Drd. Usaj Marko – tanar cercetator

Project team - slovenian partner
Prof. Miklavcic Damijan - slovenian project coordinator
Prof. Sersa Gregor – researcher
PhD Pavlin Mojca – researcher
PhD Pucihar Gorazd – researcher
Haberl Sasa – Ph.D. Student, young researcher
Usaj Marko – Ph.D. Student, young researcher

Obiectivele generale

Scopul proiectului comun de cercetare este acela de a elabora metode biofizice de cuantificare a parametrilor celulari care sunt modificati in urma aplicarii pulsurilor electrice. Aceste metode vor fi folosite pentru monitorizarea efectelor protocoalelor de electrochimioterapie asupra starii morfologice si functionale a celulelor supuse pulsurilor electrice electropermeabilizante. In plus, se doreste a se identifica si caracteriza posibilele mecanisme biologice si fizice ce stau la baza electropermeabilizarii membranelor celulare. Se urmareste de asemenea a se facilita implementarea metodei de electrochimioterapie in protocoalele clinice de tratament al tumorilor primare si metastatice in Romania.

General aims of the project

The general aim of the bilateral project was to elaborate biophysical methods to quantify the evolution of the cellular parameters which are modified due to the application of electropermeabilizing pulses. These methods are ment to monitor the effects of the electrochimiotherapeutical protocols on the morphological and functional status of the cells. Moreover, the biological and physical mechanisms resposible for electropermeabilization of the cell membrane were also under study. The project ment to prepare the implementation of the electrochimiotherapy as clinical protocol of primary and metastatic tumors in Romania.

Realizari

Colaborarea bilaterala cu partenerii sloveni, desi a durat numai 17 luni, a permis un schimb de 4 cercetatori in ambele sensuri, astfel incat fiecare grup a beneficiat de expertiza legata de specificul fiecaruia din laboratoarele partenere si s-au putut efectua experimente comune.

Discutiile si analiza datelor in laborator impreuna cu partenerii de colaborare au condus la o mai buna intelegere si interpretare a datelor obtinute si a masuratorilor efectuate.

Astfel s-a putut semnala ca masuratorile de fluiditate si polarizare generala a membranei marcate fluorescent a celulelor in suspensie sunt influentate de internalizarea fluoroforului in cursul electroporarii si valorile masurate ale fluorescentei trebuie interpretate tinand cont de acest fenomen.

S-au creat protocoale pentru masurarea fluiditatii membranare post-electroporare atat pentru observatia la microscop -single-cell (partenerul sloven), cat si pentru masuratori pe suspensii celulare (partea romana).

S-au creat deasemenea protocoale pentru electroporare cu raport optim celule porate/celule viabile, precum si pentru monitorizarea parametrului *generalized polarization* si a productiei speciilor reactive de oxigen in urma porarii celulelor din linia maligna B16-F10.

In mod curent, strategiile electrochimioterapeutice nu sunt inca implementate clinic in spitalele din Romania. Electroporarea/electropermeabilizarea ca tehnica biotehnologica este in prezent dezvoltata in Laboratorul de Biofizica si Biotehnologie Celulara din cadrul UMF Carol Davila (partenerul roman). Optimizarea protocoalelor de electropermeabiliazare pentru transferul medicamentos in linii celulare specifice, in raport cu variate boli sau cancere cutanate, reprezinta obiectul cercetarii in cadrul mai multor proiecte nationale si internationale ale laboratorului, incluzand aplicatia FP7 (INTAS Ref.N 06-1000031-10173) si reteaua Econet, la care participa si partenerii sloveni.

Colaborarea a dus deasemenea la aplicatia reusita pentru un nou proiect bilateral romano-sloven 2010-2011, intitulat **Studiu al proprietatilor electrice si statusului functional al celulelor supuse electroporarii,** care va permite continuarea cercetarilor incepute in proiectul care se incheie acum. O serie de comunicari orale si poster la conferinte internationale si nationale ale echipelor partenere confirma succesul acestei colaborari.

Abstract

Althaugh the project duration was only 17 month, the bilateral cooperation with sloven partners allowed the reciprocal exchange of 4 young researchers, so that each group benefitted of specific expertize of the partner laboratory and commune experiments could be performed.

The analysis of the data obtained together with collaboration partners led to a better understanding and interpretation of the measurements results.

It could be thus revealed that fluidity and general polarization measurements of the fluorescence labeled membranes of cells in suspension are influenced by fluorofor internalization during electroporation and that the recorded values of the fluorescence must be interpreted taking into account this phenomenon.

Protocols for monitoring the post-electroporation membrane fluidity were created for single cell observation under microscope (the sloven partener) as well as for measurements on cell suspensions (romanian partner).

There were also created protocols for optimal ratio of the porated vs. surviving cells as well as for monitoring the parameter *general polarization* and the Oxygen Reactive Species consecutive to application of electroporation pulses to malignant cells. The electrochemotherapy protocols are not yet currently implemented in hospitals in Romania; however electroporation/electropermeabilization as a biotechnological technique is presently developed in the Laboratory of Biophysics and Cell Biotechnology from Carol Davila Medical and Pharmaceutical University-Bucharest (Romanian Partner). Optimisation of protocols for drug transfer to specific cell lines using electropermeabilization in relation to various deseases and skin cancers is the research object of our group in several national and international projects, including an FP7 application (INTAS Ref.N 06-1000031-10173) and Econet network, in which are involved also our slovenian partners.

The previous collaboration led also to obtaining support for a new bilateral project untitled „**Study of the electrical properties of the electroporated cells"** for 2010-2011, allowing thus the continuation of the research project which is closing now.

A considerable number of oral and poster communications presented by both collaboration partners at national and international meetings confirms the succes of this collaboration.