TO MAGNIFICO RETTORE OF UNIVERSITA' DEGLI STUDI DI MILANO **ID CODE 5977**

I the undersigned asks to participate in the public selection, for qualifications and examinations, for the awarding of a type B fellowship at **Dipartimento di Oncologia ed Emato-Oncologia**

Scientist- in – charge: **Prof.ssa Sigismund Sara Lucia Giustina**

Gorana Jendrisek

CURRICULUM VITAE

PERSONAL INFORMATION

Surname	Jendrisek
Name	Gorana

PRESENT OCCUPATION

Appointment	Structure
Postdoctoral researcher in the lab of Prof. Di Fiore and Prof.ssa Sigismund	European Institute of Oncology

EDUCATION AND TRAINING

Degree	Course of studies	University	year of achievement of the degree
PhD (4 years)	Systems Medicine, Module Molecular Oncology	University of Milan, Italy	2022
Master (1 year)	Molecular Biology and Physiology, Module Experimental Biomedicine	Faculty of Biology, University of Belgrade, Serbia	2018
Bachelor (4 years)	Biology, Module Molecular Biology and Physiology	Faculty of Biology, University of Belgrade, Serbia	2017

REGISTRATION IN PROFESSIONAL ASSOCIATIONS

Date of registration	Association	City
2020	Associazione di Biologia Cellulare e del Differenziamento (ABCD)	Milan, Italy

FOREIGN LANGUAGES

Languages	level of knowledge
English	B2
Italian	B1
Serbian	native

AWARDS, ACKNOWLEDGEMENTS, SCHOLARSHIPS

Year	Description of award
2020-2023	Italian Association for Cancer Research (AIRC) Foundation Fellowship for Italy for the project "Dissecting the role of non-clathrin endocytosis of the EGFR in cell physiology and in breast cancer"
2018-2020	Doctoral fellowship from University of Milan and European School of Molecular medicine in the lab of Prof. Di Fiore and Prof.ssa Sigismund laboratory
2014-2017	Scholarship for the best students awarded by the Ministry of Science and Technological Development of the Republic of Serbia

TRAINING OR RESEARCH ACTIVITY

PhD thesis project: *Regulation and physiological role of non-clathrin endocytosis of the epidermal growth factor receptor*

The epidermal growth factor receptor (EGFR) plays a pivotal role in physiological cellular processes, but its aberrant activation is linked to cancer progression. Endocytosis is a critical regulator of EGFR activity. We have previously identified a novel endocytic route of the EGFR, non-clathrin endocytosis (NCE), which is activated only after stimulation with high doses of the ligand epidermal growth factor (EGF). Unlike canonical clathrin-mediated endocytosis (CME) that directs receptor mainly to recycling, NCE leads mostly to receptor degradation, restricting EGFR signaling and protecting cells from overstimulation.

The molecular characterization of the NCE pathway, led to the identification of a key functional regulator of NCE: the endoplasmic reticulum (ER)-shaping protein, reticulon-3 (RTN3). This regulator is necessary for the formation of contact sites between the plasma membrane (PM) and the ER during NCE internalization. Localized Ca^{2+} release at these contact sites induces the fission of NCE vesicles, completing the internalization process. In addition, CD147, a member of the immunoglobulin superfamily, was identified as a cargo co-internalizing with the EGFR in NCE vesicles. CD147 has been validated as a specific marker of NCE, and together with RTN3, represents an invaluable experimental tool for investigating this pathway.

In the present work, we have dissected the signaling pathway promoting Ca^{2+} release from the ER upon EGFR-NCE activation, uncovering a specific role of the phospholipase C γ2 (PLCγ2) enzyme. PLCγ2-dependent Ca^{2+} release at NCE sites occurs through the activation of the inositol trisphosphate receptor

(IP3R) on the ER membrane and is required for the fission of EGFR/CD147-positive NCE vesicles from the PM.

At the functional level, we extended the relevance of NCE to alternative EGFR ligands, beyond the EGF. Saturating doses of the two EGFR ligands, amphiregulin (AREG) and transforming growth factor-α (TGF-α), differentially triggered EGFR/CD147 internalization via NCE: while AREG efficiently activated NCE and Ca^{2+} release at the PM, TGF-α was less effective at triggering this pathway. This finding led us to hypothesize that the differential ability of alternative EGFR ligands to activate NCE could be responsible for the differences in the EGFR fate and biological output exerted by these ligands. Interestingly, stimulation of cells with another growth factor, hepatocyte growth factor (HGF), induced CD147-NCE similarly to stimulation with EGF, implying a broader role of NCE in the regulation of surface proteins.

As a model system to test the physiological relevance of NCE, we are using organoids prepared from the non-transformed breast epithelial cell line MCF10A and from primary mouse intestinal crypt and mammary gland epithelial cells. These cells are dependent on EGFR signaling for their growth and differentiation in Matrigel. Our data showed that treatment with compounds that inhibit EGFR-NCE increase growth of mice intestinal and mammary organoids.

In conclusion, we have molecularly dissected the signaling pathway leading to EGFR-NCE and expanded its relevance to alternative EGFR ligands and other growth factors. Given its crucial role in downregulating signaling and mediating growth restriction, NCE could behave as a possible tumor suppressor pathway and its regulators could represent novel targets in cancer therapy.

Master thesis project: *The effect of montelukast on inflammation in bronchial epithelial cells*

Asthma is the most common chronic respiratory disease among children. It is a complex disease resulting by a combination of genetic and environmental factors. The goal of modern asthma therapy is to establish and maintain the control of clinical manifestations of the disease over a longer period of time. However, there is high variability in the response to the asthma therapy among patients which might be due to different genetic factors. Since leukotriene receptor antagonists (LTRA) are anti-inflammatory medications widely used in asthma therapy, the aim of this research is to investigate the influence of montelukast, the mostly prescribed LTRA, on the expression of genes implicated in inflammatory response and airway remodeling, for the purpose of its use in the earlier stages of asthma therapy. The experiment was done in the bronchial epithelial cells (BEAS 2B) which were previously treated with montelukast and then the inflammation was induced by LPS. The untreated cells, the cells treated only with montelukast and with LPS were also included in the experiment. Total RNA and cell culture supernatant are prepared for further analysis. The expression levels of selected genes (TGFB1, TNF, IL-6 and MMP9) were measured by qPCR, glyceraldehyde-3-phosphate dehydrogenase is used as the internal housekeeping gene control. Level of secreted proteins were analyzed with Western Blot. The level of TGFB1 proteins were measured by ELISA.

Major finding showed that montelukast attenuates the increased expression of TNF, IL-6 and MMP9 genes after treatment with lipopolysaccharide (LPS). This effect was particularly evident on expression of the TNF and IL6 genes, which encode the key proinflammatory mediators. The expression of TGFB1 gene was decreased in cells treated only with montelukast compared with untreated cells.

Pretreatment of cells with montelukast, bearing in mind all of its anti-inflammatory effects, can potentially be used to mitigate the effects of acute inflammation, so the testing of montelukast application in the early stages of disease might also be important for improving the therapeutic approaches in asthma, as well as its more frequent application in therapy.

Internship at Weizmann institute of Science in Israel: *Regulation of signal transduction by non-coding RNAs: Investigation of effects of epidermal growth factor (EGF) signaling and transcription factor c-Myc on levels of small nucleolar RNAs (snoRNAs)*

Studies show that snoRNA levels are altered in cancer and they may play a role in tumors progression. The EGF can bind to its receptor which belongs to the superfamily of receptor tyrosine kinases. Dysregulated activation of this signaling network has been implicated in diverse types of cancer. c-Myc is an oncogenic transcription factor. In this project I investigated the effect of EGF signaling on snoRNAs levels in an attempt to identify snoRNAs that may play a role in cancer, by looking at both the AKT and MAPK pathways, and investigating the particular role of the transcription factor Myc. Verification of activation of Akt and MAP kinase pathways was done by Western blot. I established an experimental system for delineating the effects of EGF signaling and c-Myc depending on the levels of snoRNAs. The level of expression of snoRNAs was measured by qPCR. The results showed that there was no correlation between levels of expression of snoRNAs and EGF signaling but on the other hand I found that some snoRNAs are down-regulated when c-Myc is knock-down. Moreover, I was able to detect a decrease in expression of snoRNA-associated proteins.

Besides that, as the second part of my project I was working on the circErbB2 in breast cancer and pulmonary cancer cell lines in an attempt to examine the role of circErbB2. Circular RNAs are a ubiquitous class of non-coding RNAs, arising from non-linear splicing of both coding and non-coding transcripts. Exon circularization occurs when a downstream splice donor joins an upstream splice acceptor. I suspected that circErbB2 can act as a sponge for small RNAs. Specifically, I was trying to compare circErbB2 expression with linearErbB2 expression and to find circErbB2 localization. Proliferation assays were followed as well. The results showed that circErbB2 affects the level of almost all snoRNAs that were inspected.

These two parts of project were done in less than two months.

Undergraduate Research Project: *Matrix metalloproteinase 9 gene polymorphisms in asthmatic children*

I was investigating how polymorphisms in 3'UTR of gene Matrix metalloproteinase 9 can affect asthma severity in children.

PROJECT ACTIVITY

Year	Project
2022-	Postdoctoral researcher in Prof. Di Fiore's Lab, Project: Dissecting the role of non-clathrin endocytosis of the epidermal growth factor receptor in cell physiology and in breast cancer Principal Advisor: Prof. Pier Paolo Di Fiore and Prof. Sara Sigismund European Institute of Oncology, Milan, Italy
2018-2022	PhD student in Prof. Di Fiore's Lab, PhD Thesis Project: Regulation and physiological role of non-clathrin endocytosis of the epidermal growth factor receptor Principal Advisor: Prof. Pier Paolo Di Fiore and Prof. Sara Sigismund European Institute of Oncology, Milan, Italy
2017-2018	Master student in Laboratory for molecular biology, Master Thesis Project: The effect of montelukast on bronchial epithelial cells

	Principal Advisor: Dr. Aleksandra Nikolic Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia
2017	The Kupcinet-Getz International Summer Science School Research topic: Regulation of signal transduction by non-coding RNAs Principal Advisor: Prof. Yosef Yarden Weizmann Institute of Science, Rehovot, Israel
2014-2017	Undergraduate internship in Laboratory for molecular biology, Research topic: Matrix metalloproteinase 9 gene polymorphisms Principal Advisor: Dr. Aleksandra Nikolic Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia

CONGRESSES AND SEMINARS

Date	Title	Place
September 2023	ABCD 2023 - The Biennial Congress of the Italian Association of Cell Biology and Differentiation Poster presentation: Signaling circuitries regulating EGFR non-clathrin endocytosis	Paestum, Italy
September 2023	ABCD 2023 – Pre-Congress meeting Oral presentation: Signaling circuitries regulating EGFR non-clathrin endocytosis	Paestum, Italy
September 2023	EMBO Workshop When biology of endocytosis meets physics: Emerging mechanisms and functions Poster presentation: Signaling circuitries regulating EGFR non-clathrin endocytosis	Venice, Italy
June 2022	Physiological Adaptations of the Endo-Lysosomal System, Gordon Research Conference Poster presentation: Unraveling non-clathrin endocytosis: Regulation and relevance in EGFR internalization	Andover, NH, USA
June 2022	Physiology, Functions and Interactions of the Endo-Lysosomal System in Health and Disease, Gordon Research Seminar Poster presentation: Unraveling non-clathrin endocytosis: Regulation and relevance in EGFR internalization	Andover, NH, USA
March 2022	National Ph.D. Meeting Oral presentation: Unraveling non-clathrin endocytosis: Regulation and relevance in EGFR internalization	Salerno, Italy

November 2020	22nd EMBL PhD Symposium Poster presentation: Dissecting the role of non-clathrin endocytosis of the EGFR in cell physiology	Heidelberg, Germany
November 2019	The 3rd European PhD and Postdoc symposium ENABLE Poster presentation: Role of non clathrin-mediated endocytosis in CD147 regulation and cellular response	Nijmegen, Netherlands
October 2019	Cell Signaling and Intracellular Trafficking in Cancer Biology: Interplay, Targeting and Therapy Poster presentation and flash talk: Role of non clathrin-mediated endocytosis in CD147 regulation and cellular response	Turin, Italy
February 2016	LS2 Annual Meeting at the University of Lausanne Poster presentation: Matrix metalloproteinase 9 gene polymorphisms in asthmatic children	Lausanne, Switzerland

PUBLICATIONS

Jendrišek, G., Nikolić, A. and Dragičević, S. (2022) 'Inflammatory modulation of the response of bronchial epithelial cells to lipopolysaccharide with pretreatment by montelukast', *Kragujevac Journal of Science*, (44), pp. 115-125. doi:10.5937/KgJSci2244115J

OTHER INFORMATION

Part of Scientific organizing committee of The 4th European PhD and Postdoc symposium ENABLE2020/21 conference, Milano, Italy

Declarations given in the present curriculum must be considered released according to art. 46 and 47 of DPR n. 445/2000.

The present curriculum does not contain confidential and legal information according to art. 4, paragraph 1, points d) and e) of D.Lgs. 30.06.2003 n. 196.

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Place and date: Milan, 13/11/2023