Summary of Professional Accomplishments

(appendix 4b)

PhD Patrycja Przygodzka

Institute of Medical Biology
Polish Academy of Sciences, Lodz, Poland

Lodz, 2023

1. Name

Patrycja Przygodzka

formerly Patrycja Barańska

2. Diplomas, degrees conferred in specific areas of science or arts, including the name of the institution which conferred the degree, year of degree conferment, title of the PhD dissertation

2005 **PhD** in medical biology	Medical University of Lodz Faculty of Health Sciences Dissertation title: "*Role of Vascular Endothelial Growth Factor (VEGF) in the early stages of proangiogenic changes in endothelial cells*." Supervisor: prof. dr hab. Zofia Pawłowska
2001 **M.Sc. in biology** specialization: biophysics	University of Lodz Faculty of Biology Dissertation title: „*Effect of low-power laser radiation on viability and oxidative state of fibroblasts (CHO K1 line) – continuous and fractionated doses*." Supervisor: prof. dr hab. Maria Bryszewska

3. Information on employment in research institutes or faculties/departments or schools of arts

2007/07/01- present	Institute of Medical Biology Polish Academy of Sciences, Lodz, Poland Assistant professor
2020/04	The Central Clinical Hospital Medical University of Lodz, Laboratory of Respiratory Viruses, contract of mandate (SARS-CoV-2 diagnostics)
2005/09/01 - 2007/07/01	Department of Medical Biochemistry and Biophysics, Umeå University, 901 87 Umeå, Sweden Post-Doc position
2004/11/01 - 2005/09/01	Centre for Medical Biology Polish Academy of Sciences, Lodz, Poland Research Assistant
2001 - 2005	Medical University of Lodz, Faculty of Health Sciences PhD program student

4. Description of the achievements, set out in art. 219 paragraph 1 point 2 of the Act

a) Title of achievements:

„Identification of new molecular factors regulating colorectal cancer cell invasiveness"

A cycle of five scientific articles related thematically.

b) Publications included in the achievements

All articles were published after the conferment of the PhD degree.

1.

Authors **corresponding author*	**Przygodzka Patrycja***, Papiewska-Pajak Izabela, Bogusz Helena, Kryczka Jakub, Sobierajska Katarzyna, Kowalska M. Anna., Boncela Joanna	
Title	**Neuromedin U is upregulated by Snail at early stages of EMT in HT29 colon cancer cells.**	
Journal Volume/article ID	Biochimica et Biophysica Acta (BBA) - General Subjects Elsevier B.V.; Nov;1860(11 Pt A):2445-2453	
Year and type	2016	research article
Score	IF = 4,702	MNiSW = 35
DOI	10.1016/j.bbagen.2016.07.012	
Citation number, Web of Science (core collection)		21

- *I am the author of one of the research hypotheses that neuromedin U is engaged in the epithelial-to-mesenchymal transition in colorectal cancer cells.*
- *I had a leading role in experimental work: taking part in the generation of the cellular model (HT29 stable clones overexpressing Snail), characterization of the clones (cell immunofluorescence staining and confocal imaging, cell functional tests as clonogenic and anoikis assay), designing and performing neuromedin U studies (real-time PCR, ELISA).*
- *I collected and prepared material for transcriptomic analysis and I summarised and analysed the results.*
- *I had a leading role in designing and preparing a manuscript of an article, as well as in the discussion with the reviewers as a corresponding author.*

2.

Authors **corresponding author*	**Przygodzka Patrycja***, Papiewska-Pająk Izabela, Bogusz-Koziarska Helena, Sochacka Ewelina, Boncela Joanna, Kowalska M. Anna	
Title	**Regulation of miRNAs by Snail during epithelial-to-mesenchymal transition in HT29 colon cancer cells.**	
Journal Volume/article ID	Scientific Reports Springer Nature; Feb 15;9(1):2165	
Year and type	2019	research article
Score	IF = 3,998	MNiSW = 140
DOI	10.1038/s41598-019-39200-7	
Citation number, Web of Science (core collection)		20

- *I am the author of one of the research hypotheses that Snail can modulate the microRNA cargo of extracellular vesicles.*
- *I took part in the generation of the cellular model (HT29 stable clones overexpressing Snail).*

- *I optimized the microRNA isolation method, collected material for transcriptomic analysis, and summarised and analysed the results.*
- *I planned the research concerning Snail regulation of miR192/194 expression and designed and performed chromatin immunoprecipitation.*
- *I had a leading role in designing and preparing a manuscript of an article, as well as in the discussion with the reviewers as a corresponding author.*

3.

Authors **corresponding author*	**Przygodzka Patrycja***, Soboska Kamila, Sochacka Ewelina, Boncela Joanna	
Title	**Neuromedin U: A Small Peptide in the Big World of Cancer.**	
Journal Volume/article ID	Cancers MDPI; 2019, 11, 1312	
Year and type	2019	review
Score	IF = 6,126	MNiSW = 140
DOI	10.3390/cancers11091312	
Citation number, Web of Science (core collection)		14

- *I initiated the idea to review the role of NMU in cancer and I planned the article structure.*
- *I did literature research and analysed databases to find NMU expression and production regulation.*
- *I designed and prepared the manuscript.*

4.

Authors **corresponding author*	**Przygodzka Patrycja***, Sochacka Ewelina, Soboska Kamila, Pacholczyk Marcin, Papiewska-Pająk Izabela, Przygodzki Tomasz, Płociński Przemysław, Ballet Steven, De Prins An, Boncela Joanna	
Title	**Neuromedin U induces an invasive phenotype in CRC cells expressing the NMUR2 receptor.**	
Journal Volume/article ID	**Journal of Experimental & Clinical Cancer Research** BMC; Sep 7;40(1):283	
Year and type	2021	research article
Score	IF = 12,658	MNiSW = 140
DOI	10.1186/s13046-021-02073-8	
Citation number, Web of Science (core collection)		1

The article was the effect of the studies performed in the Sonata Bis 6 project.

- *I am the author of the research hypothesis, I defined the goals and assigned the tasks*
- *I decided on the methodology and most of the protocols used.*
- *I summarised and interpreted gene expression analysis, chose a cell line panel for the project, and took part in the gene expression optimization and analysis (real-time PCR). I optimized and analysed data concerning the calcium mobilization study and analysed Integrins expression (flow cytometry).*
- *I designed and had a leading role in preparing a manuscript of an article and I discussed with the reviewers as a corresponding author.*

5.

Authors **corresponding author*	**Przygodzka Patrycja***, Soboska Kamila, Sochacka Ewelina, Pacholczyk Marcin, Braun Marcin, Kassassir Hassan, Papiewska-Pająk Izabela, Kiełbik Michał, Boncela Joanna	
Title	**Neuromedin U secreted by colorectal cancer cells promotes a tumour-supporting microenvironment.**	
Journal Volume/article ID	Cell Communication and Signaling BMC; (2022) 20:193	
Year and type	2022	research article
Score	IF = 7,54	MNiSW = 140
DOI	10.1186/s12964-022-01003-1	
Citation number, Web of Science (core collection)		0

The article was the effect of the studies performed in the Sonata Bis 6 project.

- *I am the author of the research hypothesis, I defined the goals and assigned the tasks*
- *I decided on the methodology and most of the protocols used.*
- *I performed IHC of NMU in CC tissue, summarised and interpreted gene expression analysis, chose and designed a cell model for the project, performed cell immunofluorescence staining and confocal imaging, and took part in the gene expression optimization and analysis (real-time PCR).*
- *I designed and had a leading role in preparing a manuscript of an article and I discussed with the reviewers as a corresponding author.*

Scientometric information (achievement) from the year of publication

IF	**35,024**
Points by KBN/MNiSW/MEiN	**595**
Citations (ISI Web of Science Core Collection)	**57**

c) Description of the main achievements

Introduction

The colon (large intestine) is the distal part of the gastrointestinal tract, extending from the cecum to the rectum and anal canal. Colon cancer and rectal cancer are often grouped together as colorectal cancer (CRC) because they have many features in common, both usually begin as small, noncancerous polyps originating from epithelial cells of the colorectal mucosa. The majority of CRC is sporadic (90 - 95% people diagnosed at age 50 or older). Hereditary CRC is conventionally divided into two major categories: hereditary non-polyposis colorectal cancer (Lynch syndrome) and those related to polyposis syndromes including familial adenomatous polyposis (FAP) [1]. According to statistics, colorectal cancer (CRC, colorectal carcinoma) is the third in men and the second in women most frequently diagnosed among all types of cancer. The introduction of screening programs for CRC resulted in an increased number of early diagnosed colon cancer cases. Despite the introduction of disease prevention, health education, and advanced neoadiuvant or post-resection therapies, the number of deaths raised in

recent years. When detected early, CRC is curable, with survival rates of > 90% at 5 years but once the tumour is in an advanced stage and metastasis becomes clinically apparent (liver, lungs, peritoneum) the prognosis is poor and the 5-year survival is dramatically shortened to 10% [2, 3].

It remains difficult to predict clinically latent versus invasive CRC tumours and to qualify patients for most effective therapy. Understanding processes leading to cancer cell invasiveness and finding the biomarkers of worse prognosis are critical to tumour staging and therapeutic choice. There is a small number of currently used prognostic markers as MSI phenotype, *KRAS/NRAS,* and *BRAF* mutation status. Despite many reports concerning promising biomarkers which expression changes in CRC and could be clinically useful [4], searching for new prognostic and predictive factors are still needed and expected.

CRC is a heterogenic disease with distinct molecular backgrounds, mechanisms of growth and progression as well as cancer cell phenotypes in the tumour mass. Different classifications of CRC were established in order to estimate prognosis, a risk of recurrence, overall survival and decide about the most efficient therapeutic approach. Tumour-node-metastasis (TNM) staging system is the most widely used prognostic standard for CRC but it has been revised several times over the past few decades to improve its prognostic performance and treatment suggestions for patients [5]. The Consensus Molecular Subtype (CMS) is most recent classification which took into account both, tumour pathological characteristics and gene expression. It includes four subtypes: immune (CMS1), canonical (CMS2), metabolic (CMS3) and mesenchymal (CMS4) [6]. CMS1 encompassed the majority of MSI tumors with a widespread CpG hypermethylation status and the frequent occurrence of *BRAF* mutations. CMS1 is characterized by increased expression of genes associated with a diffuse immune infiltration and worse survival after relapse. CMS2 tumors displayed higher chromosomal instability (CIN) and strong upregulation of WNT and MYC downstream targets. Subtype CMS3 is characterized by low chromosomal instability, frequent occurrence of *KRAS* mutations and metabolic deregulation (i.a. glutamine and/or fatty acids). Finally, CMS4 tumors with worse overall survival, showed clear upregulation of transforming growth factor (TGF)-β signaling, angiogenesis, and the inflammatory system [6, 7]. Defined subtypes help with CRC classification but their clinical usefulness is still analyzed [8].

Researchers studying cancer progression, which is the main cause of worse survival rates, have suggested the epithelial-to-mesenchymal transition (EMT) as a crucial process in invasive phenotype acquirement by cancer cells. EMT is a reversible process important i.a. in embryonic development and tissue remodeling [9, 10] which was adopted by cancer cells as a mechanism leading to cancer progression [11]. Changes in genes expression during EMT induce modulation of cancer cell phenotype. Epithelial cells with apical-basal polarity and high cell-cell adhesion acquire mesenchymal features including spindle-like morphology, lost polarity, cell-cell dissociation, and enhanced motility. EMT is induced and controlled by a range of transcription factors such as Snail, Twist and Zeb family. Morphological and functional changes in cancer cells are related to decreased epithelial markers

expression (i.e. E-cadherin, claudins, occludin) and an increase in expression of mesenchymal markers (i.e. vimentin, fibronectin, N-cadherin). For a long time, EMT was perceived as a single transition between epithelial and mesenchymal states. However, there have been many observations, including our own data presented below, which verified this view. Now it is accepted that EMT is a dynamic transition within the spectrum of intermediate states with metastable points in the process, which enable cancer cells adaptation to the microenvironment. This epithelial-mesenchymal plasticity with EMT and the opposite MET (mesenchymal-epithelial transition) became a new potential therapeutic target and has started an intensive search for universal biomarkers (proteins, RNA, microRNA) of cancer cell metastatic potential [12]. Combining classical anti-tumour therapy with epithelial-mesenchymal plasticity inhibitors gives hope for more effective cancer treatment and a lower risk of disease recurrence.

Many years of cancer cell plasticity research showed that tumour progression has to be investigated in the context of a whole tumour microenvironment (TME). Tumour niche consists of extracellular matrix, fibroblasts, immune cells and endothelial cells of blood and lymphatic network [13]. Those components with their interactions should be intensively analysed beside widely studied cancer cells, and the processes connected with this complicated environment are recently most interesting in cancers, including CRC.

Results presented in the articles included in the achievements enrich incomplete knowledge concerning pro-metastatic processes in the TME and molecular mechanisms leading to CRC progression. They are presented below under two main issues.

Transcriptomic and functional changes in colorectal cancer cells at the early stage of EMT *(article 1)*

The research was performed within the Maestro project in which I was involved since 2012. The main goal of the project was to detect changes in CRC cells caused by overexpression of *Snail*. Snail is a transcription factor already known as a molecular inducer of epithelial-to-mesenchymal transition process leading to invasive phenotype acquirement by CRC cells as well as other cancer cell types. As an experimental model we used HT29, CRC cell line with stable overexpression of *Snail*. HT29 cells have epithelial phenotype with low endogenous *Snail* expression and low invasiveness. Clones with various *Snail* overexpression level (HT29-Snail) as well as control clones (HT29-pcDNA) were generated by clonal selection with geneticin. We observed that HT29-Snail clones acquired mesenchymal features such as spindle-like morphology, cell-cell dissociation, decreased expression of genes coding epithelial markers (E-cadherin, claudin-1), and an increase in expression of genes coding a mesenchymal marker, vimentin. Changes in clones' proliferation, adhesion, and migration abilities showed that *Snail* overexpression induced EMT in HT29 cells. It validated our experimental model and its utility in functional changes analysis of CRC cells at the early stages of EMT.

Next, we isolated total RNA from selected HT29 clones (HT29-pcDNA and HT29-Snail) and performed transcriptomic analysis using microarrays. **We identified 541 differentially expressed genes. Among those, 340 genes were down-regulated, while 201 genes were upregulated as a result of *Snail* overexpression. Ingenuity Pathway Analysis software, which correlates transcriptomic changes with cell functions, showed that *Snail* overexpression in CRC cells mainly influenced cell motility, what we confirmed experimentally, as well as cell viability. However, global transcriptomic and functional changes indicated that Snail upregulation results in incomplete phenotype conversion, up to the intermediate epithelial state;** cells express mesenchymal markers and become more motile and invasive but still proliferate and maintain some epithelial features. These observations confirmed rare reports that **the EMT process in CRC cells should be perceived as a spectrum of intermediate states between epithelial and mesenchymal. Thus it is not the cancer cell phenotype but the processes which control cancer cell plasticity that should be perceived as new potential therapeutic targets.** Cancer cell heterogeneity in the tumour is the main cause of cancer progression despite applied therapy.

Further, we analysed transcriptomic data according to the most deregulated genes upon *Snail* overexpression. Besides genes already correlated with EMT as *FN1* coding fibronectin or *TNC* coding tenascin C, we detected many new interesting genes coding glycoproteins or collagens, which previously were not correlated with CRC cells invasiveness.

We focused on *NMU* coding small neuropeptide, neuromedin U (NMU), not reported before in the context of CRC and rarely correlated with other cancer types progression. The knowledge concerning *NMU* expression, peptide synthesis, and secretion was limited. The studies of NMU action through its receptors NMUR1 and/or NMUR2 beside the central nervous system were difficult to perform because of low endogenous expression of the receptors and lack of validated antibodies. Thus we had to perform basic analysis concerning NMU. **We confirmed increased *NMU* expression in HT29-Snail clones which reflected *Snail* overexpression level. We detected mRNA coding NMU and NMU peptide in the culture medium from HT29-Snail clones. We showed for the first time that CRC cells with induced EMT (by *Snail* overexpression) and increased mobility, produce and secrete NMU and express its receptor.**

All above observations were further investigated in the following projects.

(article 2)

MicroRNAs with their regulatory functions became new interesting molecules in the field of cancer progression and metastasis research [14]. As a next step of our research we searched for changes in microRNA levels that were correlated with the early EMT process induction and growing CRC cell invasiveness. We used a previously generated model of HT29 cells overexpressing *Snail* and performed microRNA analysis using *miRCURY LNA™ microRNA Array 7th Gen* (Exiqon), which contains capture probes targeting all microRNAs for human, mouse or rat registered in the miRBase 18.0 as well as microRNA from viruses and SNORDs. We showed **that EMT induced by *Snail* overexpression**

did not alter the relative expression levels of genes coding the key miRNA processing enzymes Drosha and Dicer so did not change the global efficiency of microRNA processing. Nevertheless, *Snail* expression significantly triggered changes in individual microRNA levels. Performed array analysis showed deregulation of several dozen microRNAs. MiR-205, let-7i, and SNORD13 were found to be particularly overexpressed, while miR-192 and miR-194 were the most reduced. Increased miR-205 and let-7i expression was confirmed by real-time PCR. It suggested their involvement in the process increasing CRC cells invasiveness and gave us arguments in the discussion about the role of miR-205 and let-7i in cancer progression which is ambiguous. The molecules are proposed as tumour suppressors or tumour promotors depending on cancer type [15-17]. Observed SNORD13 increase in CRC cells with *Snail* overexpression was interesting. Although it was not investigated by us further, recent reports about the role of small nucleolar RNAs in metastasis [18] suggest that future studies concerning this molecule could give interesting results.

MiR-192 and miR-194 were shown to have inhibitory effect on tumour progression and were downregulated in HT29-Snail clones. Snail is known as a transcriptional repressor, so we asked if its overexpression can directly repress the production of miR-192 and miR-194. We showed that *Snail* silencing caused an increase in microRNAs levels and we revealed Snail affinity for the pri-miR-192/194 promoter region by chromatin immunoprecipitation. **Thus, we conclude for the first time that Snail can directly bind microRNA promoter and repress the expression of microRNA engaged in tumour suppression in CRC cells.**

Transcriptomic and microRNA analyses of CRC cells at the early stage of EMT provided huge amount of data which we combined as a consistent list of deregulated microRNA with changes in their target genes. In that way we showed regulatory potential of microRNA during EMT.

To accomplish characteristic of the CRC cells with Snail-induced EMT, we investigated how *Snail* expression affects the microRNA transported in extracellular vesicles (EVs) released from HT29 cells. Purified and characterized EVs were used for mRNA isolation and microRNA detection by next-generation sequencing (NGS). Complete data are presented in the article not included in the current achievement but analysis of **the cargo of EVs released from clones overexpressing *Snail* showed that the pattern of detected intracellular changes in microRNA levels were reflected in the EVs content.** EVs known for their role in intercellular communication can fulfil this task via microRNA transport.

Described results inspired following studies and projects concerning functions of glycoproteins, collagens and neuromedin U in CRC cells with induced EMT and increased invasiveness.

Role of neuromedin U in the colorectal cancer microenvironment.

(article 3)

After microRNA analysis, I revisited gene expression deregulations detected in CRC cells with the induced EMT process. The role of neuromedin U in CRC progression seemed to be very interesting and the results of preliminary studies became the foundation of the project proposal Sonata Bis 6 (NSC)

entitled: "Neuromedin U, new potential regulator of colorectal cancer metastasis mechanisms", which received financial support that enabled continuation of the research. Because of growing number of reports suggesting NMU as an important peptide in cancer progression, we summarized knowledge concerning this subject in the review. We described *NMU* expression, peptide synthesis, secretion and processing, as well as, the types, distribution and functions of classical (NMUR1, NMUR2) and alternative (NTSR1/GHSR1b) NMU receptors which belong to G-protein-coupled receptors family (GPCR). Neuromedin U is a conservative peptide with expression found in many species in various isoforms. In humans, it is a 25- amino acid long peptide widely conserved between organisms, with emphasis on the amidated C-terminal pentapeptide (-Phe-Arg-Pro-Arg-Asn-NH2). NMU action was studied mainly in the central nervous system but, as we mentioned in the article, there was a lack of many basic information as i.a. complicated steps of NMU production regulation. **The main and the most interesting part of the review is the summary of NMU and NMU receptors expression, and function in cancer diseases**. We found that reports described the level of *NMU* expression and peptide secretion but without data concerning NMU forms, NMU activity and target cells in the tumour niche. We found that most of the NMU receptors studies were performed using cell lines model with receptors overexpression so they concluded about the mechanism of receptor activation, but not about the actual activity of the receptors in cancer cells. Additionally, reports showed that there were some difficulties in NMU receptor identification on the protein level, that was informative and helped with further work planning in the project. We found that in most cancer types, except for oral and esophageal cancers, increased *NMU* expression was related to disease progression and *NMUR1* and *NMUR2* expression was diversified among tumour types. In spite of the growing interest in NMU as a peptide involved in cancer progression, there were no reports concerning the role of NMU in CRC.

(article 4)

The work plan of the Sonata Bis 6 project assumed verification of two hypothesis. First, that CRC cells are activated by secreted NMU and second, that other, noncancerous cells present in the tumour microenvironment and characterized by NMU receptor expression, are potential NMU targets. First part of the research was summarized in the fourth presented article. As it was a pioneer study, we started with gene expression analysis in CRC tissues using data collected in The Cancer Genome Atlas (TCGA). It was done in collaboration with dr Marcin Pacholczyk, adjunct in the Department of Systems Biology and Engineering, Silesian University of Technology (Gliwice, Poland). We showed **increased *NMU* expression in CRC tissues in comparison to normal adjacent tissue (NAT), even when isolated at the early stage of the disease. Additionally, a high *NMU* level in CRC tissue was related to a worse prognosis. *NMUR1* expression was significantly lower and *NMUR2* expression was significantly higher in tumour tissue when compared to NAT.** This unexpected result determined our further studies. Moreover, we observed lower *CDH-1* expression (a marker of cell differentiation) and higher *MMP-1* level (mediator of primary tumour invasiveness) in tissues with high *NMUR2* expression**. Increased *NMU* and *NMUR2* expression in human tissues correlated with more perineural**

invasions, related to worse prognosis. As data collected in TCGA show gene expression in the whole tumour tissue, we could not conclude about *NMU* expression in particular cell types present in CRC tissue, thus for subsequent detailed molecular studies we used CRC cell line panel.

NMU expression was identified in six, well-characterized, CRC cell lines representing distinct genotypes and phenotypes (Caco-2, SW480, SW620, HCT15, HCT116, and HT29) in contrast to normal epithelial cell line used as a control (CCD 841 CoN). Results confirmed heterogeneity of chosen cell lines which expressed different levels of *NMU* and produced and secreted various levels of the peptide. We identified cell lines characterized by low *NMU* expression, where the method used was not sufficient to identify protein (Caco-2, HT29, SW620) and cell lines with high *NMU* expression, where we identified NMU in cell lysates as well as in culture medium (HCT15, HCT116, SW480) and we confirmed secretion of NMU by CRC cells. NMU highly secreted to the culture medium was analysed by mass spectrometry. We showed for the first time that **NMU is secreted as 174-AA and 158-AA precursors. This was new and important information as the process of synthesis and processing of NMU was not described before and is still not fully elucidated.** Another achievement of the presented paper was **the identification of NMU as a cargo of extracellular vesicles, known for their activity in cellular communication during cancer progression.** The importance of this observation remain uncovered yet.

Next, we asked if CRC cells are the targets of NMU secreted by cancer cells. To answer that, we analysed NMU receptors expression on CRC cells. We showed that HCT116 cells expressed *NMUR1*, Caco-2, HT29, SW480 expressed *NMUR2* and HCT15 cells did not express any of the receptors. Additionally, cell lines SW480 and SW620 are potentially able to form alternative NMU receptor, NTSR1/GHSTR1b. These data point again to the heterogeneity of CRC cells. As there was no data about *NMUR1* and *NMUR2* expression regulation in cancer cells, we focused on this issue and **showed that both receptors are regulated by DNA methylation. It is possible then, to "switch on" and "switch off" receptors' expression by cancer cells and by this enable their adaptation to the variable tumour microenvironment.** We showed that CRC cell lines with NMU receptor expression represent cancer molecular subtypes CMS3 and CMS4, with a worse prognosis which are difficult to treat. Our expression data showed that some types of CRC cells might potentially respond to secreted NMU and autocrine activation is possible. NMURs activation was previously investigated using cell lines with exogenous receptor overexpression. **We showed for the first time NMU/NMUR2 signal activation in CRC cells with endogenously expressed *NMUR2* by detection of intracellular calcium mobilization and ERK1/2 kinase phosphorylation.** The phosphorylation of ERK (p-ERK) is acknowledged as a common endpoint measurement for the activation of GPCR receptors [19]. Nevertheless, because of low levels of NMURs genes expression (typical of GPCR receptors) we were not able to detect receptor activation by CRC cells treatment with synthetic NMU-9. Only the usage of more stable NMURs agonists let us identify activation of CRC cells. NMU receptor agonists and their stability characterization were crucial in this part of the study and were done in collaboration with prof. Steven

Ballet from Vrije Universiteit Brussel, Belgium. Prof. Steven Ballet's group helped us to identify problems with peptide activity and delivered us, well-characterized NMURs agonists (NMU-8 analogues), with enhanced affinity and potency for NMU receptors and with increased proteolytical stability [20]. We concluded that a high local concentration of stable peptides is crucial for NMURs activation. As we confirmed NMUR2 activation on CRC cells, we analysed whether NMU action affects the phenotype of cancer cells. We generated and used a model of HT29 and Caco-2 clones with stable overexpression of *NMU*. This model allowed us to analyse CRC cells expressing *NMUR2* and continuously activated by secreted NMU. **Selected CRC clones with *NMU* overexpression had increased migratory and invasive potential and activated ERK1/2 kinase pathway**, what indirectly showed the NMUR2 activation. Mobility and invasion of cancer cells depends strongly on cancer cell interaction with extracellular matrix. Hence we analysed the expression of genes coding integrins, surface receptors actively engaged in cancer progression [21]. **We found that continuous activation of CRC cells by NMU increased the expression of genes coding αV, α2, α6, β1, β4, β6 integrin subunits, which are active in the cancer progression.**

To sum up, our data confirmed that increased production and secretion of NMU by CRC cells causes autocrine activation of cancer cells through NMUR2 and changes their phenotype toward more invasive.

(article 5)

As a next step of the project, we decided to validate previously reported *NMU* expression changes identified by TCGA in CRC tissues on the protein level. In order to do that, NMU level was detected in commercially available human CRC tissue samples by immunohistochemistry (IHC). **A significant increase in NMU staining was detected mainly in low-grade CRC tissues (g1).** The differences decreased with the disease progression.

TCGA data set analysis showed a shorter overall survival of patients with high *NMUR1* expression. In this group, we identified more lymphatic metastasis and increased expression of *VEGFC* (vascular endothelial growth factor type C) which induces lymphatic vessels. This findings were unexpected, as *NMUR1* expression were decreased in CRC tissue compared with NAT, which suggests a tumour-suppressive rather than a tumour-supportive role for the receptor. It got our interest, especially in the light of inconsistent reports concerning the role of NMU receptors in other cancer types (*article 3*). As *NMUR1* expression was reported in other, noncancerous cells present in the tumour microenvironment, we analysed TCGA data and search for molecular markers of tumour niche cells in CRC. **We observed that increased expression of *NMUR1* correlates with an elevated expression levels of i.a. endothelial cells (EC), macrophages and platelets.** We hypothesized that NMU released by CRC cells influences cancer cells and other *NMUR1*-positive cells present in the TME. Data collected in TCGA show gene expression in the whole tumour tissue, so to conclude about *NMUR1* expression in particular cell types present in CRC tissue we employed cellular models. We used TDMs (THP-1-derived macrophages) obtained by THP-1 cells differentiation and MDMs (monocytes-derived

macrophages) obtained by peripheral blood mononuclear cells (PBMCs) differentiation. HMEC-1 cell line was used as a model of microvascular endothelial cells and platelets were isolated from concentrates of human blood platelets of healthy donors. We confirmed experimentally *NMUR1* mRNA and protein presence in all tested cell types. Cells were treated with synthetic NMU-9 or with conditioned medium from *NMU* overexpressing CRC cells. To verify NMUR1 activation we analyse ERK1/2 kinase phosphorylation, as it was done in case of CRC cells, and we showed receptor activation in NMU-treated endothelial cells and macrophages. **We showed that noncancerous cells potentially present in the tumour microenvironment are able to react on CRC cells secreted NMU stimulation.** Cell phenotype and functions analysis after NMU treatment was not studied before so we focused on key processes ongoing in tumour niche as cytokines secretion, chemotaxis, migration and angiogenesis. **We observed that macrophages migrate significantly faster towards medium containing NMU-9 suggesting the chemotactic role of NMU in the tumour environment** which potentially increases macrophages number in the TME. Additionally, NMU **promoted motility and induced polarization of macrophages into tumour supporting M2 phenotype (CD206 marker presence), and changed the profile of secreted cytokines, i.a. increased CCL-2 and CXCL-12 levels which promote cancer progression. Our results let us conclude that NMU is able to promote CRC progression by modulation of the presence and phenotype of macrophages.** Thereby we join to the discussion about controversies concerning macrophage role in CRC progression [22].

To determine how NMU might indirectly modulate CRC cells via cytokines produced by macrophages, **we treated CRC cells (HCT116 cell line) with conditioned medium from NMU-treated macrophages. As the effect we observed increased motility of HCT116 cells. It means that by secreting NMU CRC cells promote tumour supportive microenvironment through autocrine action and paracrine activation of macrophages.**

Endothelial cells in the tumour niche (TEC, tumour endothelial cells) create dysfunctional endothelium [23] which provide nutrients to the tumour, induce angiogenesis and support cancer cell intravasation. Vessels near the tumour enables matastasis. We showed that **NMU treated endothelial cells gained features of dysfunctional endothelium, increased the expression of the TEC markers, insulin-like growth factor-binding protein 7 (IGFBP-7) and vimentin and changed a profile of secreted cytokines. In the group of detected cytokines with elevated secretion (CCL-2, CCL-5, CXCL-10, G-CSF i GM-CSF, ICAM-1 oraz IL-6) there was proangiogenic ICAM-1, CCL-2 which determines interaction with macrophages and CCL-5 which together with CCL-2 determines interaction cancer cells. Moreover, functional experiments showed that NMU induces endothelial cell motility and promotes microtube formation in 3D cultures.**

To summerise, our results showed that NMU can act through macrophages and endothelial cells and its activity is used by CRC cells to create pro-metastatic tumour microenvironment.

Inspite of the knowledge concerning increased expression of *NMU* in many cancer types and the presence of NMU receptors on cells characteristic for tumour niche [24] our studies of the role of neuromedin U in the tumour microenvironment were novel.

Still, our observations were basic and it is too early to define NMU or its receptors as CRC progression markers. It is also still not clear if the next findings reveal NMU/NMUR pathway as a therapeutic target. However, our results, recent reports from other cancers and new experimental methods development encourage further studies.

Results and observations obtained within the Sonata Bis 6 project were summarized in the PhD thesis of dr Kamila Soboska and MSc Ewelina Sochacka. Our data inspired further ideas concerning the study of the role of NMU in CRC which I included in the new grant proposal titled: "Prognostic potential of neuromedin U and its receptors in colorectal cancer, importance in the metastasis". The project received a recommendation for funding within Opus21 (NSC) and has started in September 2022. I will perform *in vivo* experiments in mouse models, analysis of human CRC tissue samples and lymphatic endothelium to verify the hypothesis that NMU and its receptors stimulate CRC invasiveness and that they are good candidates for prognostic markers in CRC

Summary of scientific achievement:

- Generation and characteristic of cellular model of CRC cells with induced EMT by stable Snail transcription factor overexpression (HT29-Snail)
- As a result of transcriptomic analysis of HT29-Snail clones with induced EMT and enhanced invasiveness:

- we identified 541 differentially expressed genes, 340 genes were down-regulated, while 201 genes were upregulated as a result of *Snail* overexpression. Identified transcriptomic changes mainly influenced cell motility and cell viability.
- we showed that global transcriptomic and functional changes detected in HT29-Snail clones indicated that Snail upregulation results in incomplete phenotype conversion, up to the intermediate epithelial state.
- we concluded that EMT process in CRC cells should be perceived as a spectrum of intermediate states between phenotypes. Thus it is not the cancer cell phenotype but the processes which control cancer cell plasticity that should be perceived as new potential therapeutic targets.
- we confirmed increased *NMU* expression in HT29-Snail clones which reflected *Snail* overexpression level. It was shown for the first time that CRC cells with induced EMT and increased mobility (by *Snail* overexpression), produce and secrete NMU and express its receptor, NMUR2.

- As a result of microRNA analysis in HT29-Snail clones with induced EMT and enhanced invasiveness:

- we showed that EMT induced by *Snail* overexpression did not alter the relative expression levels of genes coding the key miRNA processing enzymes Drosha and Dicer so did not change the global efficiency of microRNA processing.

- we showed that *Snail* overexpression significantly triggered changes in individual microRNA levels. Performed array analysis showed deregulation of several dozen microRNAs. MiR-205, let-7i, and SNORD13 were found to be particularly overexpressed, while miR-192 and miR-194 were the most reduced.

- for the first time we showed that Snail can directly bind microRNA promoter and repress the expression of microRNA engaged in tumour suppression in CRC cells.

- transcriptomic and microRNA analyses of CRC cells at the early stage of EMT provided huge amount of data which were summarized as a consistent list of deregulated microRNA with changes in their target genes. In that way we showed regulatory potential of microRNA during EMT.

- Knowledge concerning NMU peptide was reviewed. NMU expression, peptide synthesis, secretion and processing, as well as, the types, distribution and functions of classical (NMUR1, NMUR2) and alternative (NTSR1/GHSR1b) NMU receptors. For the first time prepared the summary of NMU and NMU receptors expression, and function in cancer diseases.

- NMU role in CRC cells activation was analysed and as a result:

- we showed increased *NMU* expression in CRC tissues in comparison to normal adjacent tissue. Additionally, a high *NMU* level in CRC tissue was related to a worse prognosis. *NMUR1* expression was significantly lower and *NMUR2* expression was significantly higher in tumour tissue when compared to normal adjacent tissue.

- *NMU* and NMU receptors expression and presence was described in a CRC cell line panel

- we showed for the first time that NMU is secreted as 174-AA and 158-AA precursors.

- we identified NMU as a cargo of extracellular vesicles, known for their activity in cellular communication during cancer progression.

- we showed that both NMU receptors are regulated by DNA methylation. It is possible then, to "switch on" and "switch off" receptors' expression by cancer cells and by this enable their adaptation to the variable tumour microenvironment.

- we showed for the first time NMU/NMUR2 signal activation in CRC cells with endogenously expressed *NMUR2* by detection of intracellular calcium mobilization and ERK1/2 kinase phosphorylation.
- It was observed that selected CRC clones with *NMU* overexpression had increased migratory and invasive potential
- It was found that continuous activation of CRC cells by NMU increased the expression of genes coding αV, α2, α6, β1, β4, β6 integrin subunits, which are active in the cancer progression.
- we confirmed that increased production and secretion of NMU by CRC cells causes autocrine activation of cancer cells through NMUR2 and changes their phenotype toward more invasive.

- NMU role in activation of noncancerous, potentially present in tumour niche cells was analysed and as a result:

- we showed activation of NMUR1 in NMU-treated endothelial cells and macrophages. It was concluded that noncancerous cells potentially present in the tumour microenvironment are able to react on CRC cells secreted NMU stimulation.
- we showed that NMU is a chemoattractant for macrophages, promotes their motility and induces polarization of macrophages into tumour supporting M2 phenotype (CD206 marker presence), and changed the profile of secreted cytokines, i.a. increased CCL-2 and CXCL-12 levels which promote cancer progression.
- we showed that by secreting NMU CRC cells promote tumour supportive microenvironment through autocrine action and paracrine activation of macrophages.
- we observed that NMU treated endothelial cells gained features of dysfunctional endothelium, increased the expression of the TEC markers, insulin-like growth factor-binding protein 7 (IGFBP-7) and vimentin and changed a profile of secreted cytokines: CCL-2, CCL-5, CXCL-10, G-CSF i GM-CSF, ICAM-1 and IL-6
- functional experiments showed that NMU is a proangiogenic factor, induces endothelial cell motility and promotes microtube formation in 3D cultures.

- Reported data concerning NMU activity in CRC microenvironment are interesting also in the context of other cancers

d) Description of other achievements

Endothelial cells functions in physiologic and pathologic angiogenesis

From 2001 to 2005, after my MSc at the University of Lodz (biology, biophysics), I was in the PhD program at the Medical University of Lodz, Department of Molecular and Medical Biophysics under the supervision of prof. Zofia Pawlowska. My doctoral research was made in the frame of the Centre of Excellence in Molecular Medicine MolMed. I was also involved in many other ongoing projects in the Department under the leadership of prof. Czeslaw S. Cierniewski. My main interests concerned then endothelial cell functions and mechanisms connected with angiogenesis process i.e. new vessel formation under physiological and pathological conditions. I analysed adhesion, migration and capillary structure formation assays, particularly in the context of tumour angiogenesis which enables tumour growth. **Using an innovative method of global proteomic analysis we described changes in the proteome of endothelial cells stimulated with vascular endothelial growth factor (VEGF) to form new vessels.** VEGF is a key factor inducing angiogenesis leading to tumour vascularization. Identification of mechanisms induced in EC during this process is crucial in the development of new therapies inhibiting tumour progression. We described significant changes in protein content after pro-angiogenic activation of EC, between other, increased levels of proteins from heat shock protein family and integrins. Results were summarised and published in Proteomics and Cancer Genomics & Proteomics. Obtained data were the basis of my PhD dissertation titled: "Role of Vascular Endothelial Growth Factor (VEGF) in the early stages of proangiogenic changes in endothelial cells". After the conferment of PhD degree I continued research concerning endothelial cells in the lab of prof. Czeslaw S. Cierniewski. We confirmed VEGF-D, growth factor released by tumour endothelial cells, as an angiogenesis inducer. Searching for factors inducing angiogenesis determined by inflammation we identified NFAT-2 / Cox-2 pathway activation which promotes angiogenesis and changes integrins expression profile.

Beside angiogenesis we got interested in vascular degeneration in diabetic retinopathy which is a result of NMDA receptors activation. Matrin 3, described as a nuclear matrix protein, currently confirmed RNA and DNA binding protein [25] was suggested as important regulator of NMDA-induced neurons death. Because of NMDA receptors expression on endothelial cells we tested matrin 3 role in those cells. We showed that it induces endothelial proliferation and silencing of matrin 3 expression induces necrosis of ECs.

Articles concerning this part of research, published before the conferment of PhD degree.

- Pawłowska Z., Jerczyńska H., Szemraj J., **Barańska P.**, Swiątkowska M., Cierniewski C.S. Natriuretic peptides reduce plasminogen activator inhibitor–1 expression in human endothelial cells. Cellular & Molecular Biology Letters, 7(4), 1153-1157 (2002); PMID: 12511982

- **Barańska P**., Jerczyńska H., Pawłowska Z. Czynnik wzrostu śródbłonka naczyń - budowa i funkcje. Postępy Biochemii, 51(1), 12-21 (2005); PMID: 16209337

- Pawłowska Z., **Barańska P.**, Jerczyńska H., Koziołkiewicz W., Cierniewski C.S. Heat shock proteins and other components of cellular machinery for protein synthesis are up-regulated in vascular endothelial cell growth factor-activated human endothelial cells. Proteomics, 5(5), 1217-1227 (2005); doi:10.1002/pmic.200400983

- Jerczyńska H., **Barańska P.**, Walkowiak B, Koziołkiewicz W, Pawłowska Z. Growth of endothelial cells at surfaces of selected biomaterials. Engineering of Biomaterials, 43-44, 21-24 (2005).

- **Barańska P.**, Jerczyńska H., Pawłowska Z., Koziołkiewicz W., Cierniewski C.S. Expression of integrins and adhesive properties of human endothelial cell line EA.hy 926. Cancer Genomics & Proteomics, 2(5), 265-270 (2005); PMID: 31394624

- Komorowski J., Jerczyńska H., Siejka A., **Barańska P.**, Ławnicka H., Pawłowska Z., Stępień H. Effect of thalidomide affecting VEGF secretion, cell migration, adhesion and capillary tube formation of human endothelial EA.hy 926 cells. Life Sciences, 78, 2558–2563 (2006); doi:10.1016/j.lfs.2005.10.016

Articles concerning this part of research, published after the conferment of PhD degree.

- Papiewska-Pająk I., Boncela J., **Przygodzka P.**, Cierniewski C.S. Autocrine effects of VEGF-D on endothelial cells after transduction with AD-VEGF-D(DeltaNDeltaC). Experimental Cell Research, Apr 1;316(6), 907-914 (2010); doi: 10.1016/j.yexcr.2010.01.014; PMID: 20096685

- Mena M.P., Papiewska-Pająk I., **Przygodzka P.**, Kozaczuk A., Boncela J., Cierniewski C.S. NFAT2 regulates COX-2 expression and modulates the integrin repertoire in endothelial cells at the crossroads of angiogenesis and inflammation. Experimental Cell Research, Jun 10;324(2), 124-136 (2014); doi: 10.1016/j.yexcr.2014.03.008

- **Przygodzka P.**, Boncela J., Cierniewski C.S. Matrin 3 as a key regulator of endothelial cell survival. Experimental Cell Research, Apr 1;317(6), 802-811 (2011); doi:10.1016/j.yexcr.2010.12.009

Role of serpins in cell functions

After the conferment of the PhD degree, I began my post-doctoral fellowship in the Department of Medical Biochemistry and Biophysics, Umeå University, Sweden, in the group of Prof. Tor Ny. My scientific activity during the post-doctoral training is described below in subsection 5 of this Summary. My scientific activity during the fellowship concerned functions of serpins which belong to the group of serine proteases inhibitors with various roles. It started my engagement in the serpins studies.

After my post-doctoral fellowship, I went back to the Institute of Medical Biology PAS and I was employed as an adjunct in the Cellular Proteomics Laboratory. I was involved in numerous projects realized in the group of prof. Czeslaw S. Cierniewski (mainly by dr Joanna Boncela). **Our studies revealed the functions of PAI-1 and PAI-2 in activity of endothelial cells and activity of proteosome**, complex degrading enzymes and regulatory proteins.

Articles concerning this part of research

- **Przygodzka P.**, Ramstedt B., Engel T., Larsson G., Wilczynska M. Bomapin is a redox-sensitive nuclear serpin that affects responsiveness of myeloid progenitor cells to growth environment. BMC Cell Biology, 11, 30 (2010); doi: 10.1186/1471-2121-11-30

- Boncela J., **Przygodzka P.**, Papiewska-Pajak I., Wyroba E., Osinska M., Cierniewski C.S. Plasminogen activator inhibitor type 1 interacts with alpha3 subunit of proteasome and modulates its activity. Journal of Biological Chemistry, Feb 25;286(8), 6820-6831 (2011); doi: 10.1074/jbc.M110.173781; PMID: 21135093

- Boncela J., **Przygodzka P.**, Papiewska-Pająk I., Wyroba E., Cierniewski C.S. Association of plasminogen activator inhibitor type 2 (PAI-2) with proteasome within endothelial cells activated with inflammatory stimuli. Journal of Biological Chemistry, Dec 16;286(50), 43164-43171 (2011); doi: 10.1074/jbc.M111.245647; PMID: 21976669

- Boncela J., **Przygodzka P.**, Wyroba E., Papiewska-Pająk I., Cierniewski C.S. Secretion of SerpinB2 from endothelial cells activated with inflammatory stimuli. Experimental Cell Research, May 1;319(8), 1213-1219 (2013); doi: 10.1016/j.yexcr.2013.02.018

Epithelial-mesenchymal transition process studies.

Results of the project concerning EMT and described in the achievements presentation were continued in various contexts. Generated model of HT29-Snail clones together with analogical model of melanoma cells overexpressing *Snail* were used to show that lumican (small glycoprotein) decreases invasive melanoma cells migration by inhibition of metalloproteinase 14 gene expression (*MMP-14*).

EMT analysis inspired studies of endothelial-mesenchymal transition (EndoMT) which is involved in cancer progression and fibrotic diseases and is regulated by the factors inducing EMT. In one of the papers we described *Snail* expression regulation by MRTF (myocardin-related transcription factor) in endothelial cells what regulates EndoMT induction.

Articles concerning this part of research

- Stasiak M., Boncela J., Perreau C., Karamanou K., Chatron-Colliet A., Proult I., **Przygodzka P.**, Chakravarti S., Maquart F.X., Kowalska M.A., Wegrowski Y., Brézillon S. Lumican Inhibits SNAIL-Induced Melanoma Cell Migration Specifically by Blocking MMP-14 Activity. PLoS One, Mar 1;11(3):e0150226 (2016); doi: 10.1371/journal.pone.0150226

- Sobierajska K., Ciszewski W. M., Macierzynska-Piotrowska E., Klopocka W., **Przygodzka P.**, Karakula M., Pestka K., Wawro M.E. and Niewiarowska J. The New Model of Snail Expression Regulation: The Role of MRTFs in Fast and Slow Endothelial–Mesenchymal Transition. International Journal of Molecular Sciences, 21, 5875 (2020); doi:10.3390/ijms21165875

Analysis of secretion and a cargo of extracellular microvesicles released by cancer cells.

Extracellular vesicles secretion, cargo and functions studies in cancer progression are very actual. Thanks to the collaboration with dr Izabela Papiewska-Pająk I was involved in the studies of EVs released by CRC cells. It was shown that MC38 cells with induced EMT release EVs with increases amount of glypican-1, glycoprotein which is a marker of CRC. Transport of glypican-1 could help with a new cancer niche establishment. Reports suggesting potential role of EVs in tumour microenvironment modulation and new cancer niche formation inspired the analysis of microRNA content of EVs released by HT29-Snail clones. Some of the results were described in the achievement presentation. Analyses confirmed that EVs released by cancer cells with induced EMT have changed microRNA cargo and in vivo analyses showed that they have pro-inflammatory potential.

Articles concerning this part of research

- Papiewska-Pajak I., Krzyzanowski D., Katela M., Rivet R., Michlewska S., **Przygodzka P.**, Kowalska M.A. and Brézillon S. Glypican-1 Level Is Elevated in Extracellular Vesicles Released from MC38 Colon Adenocarcinoma Cells Overexpressing Snail. Cells, 9, 1585 (2020); doi:10.3390/cells9071585

- Papiewska-Pajak I., **Przygodzka P.**, Krzyzanowski D., Soboska K., Szulc-Kiełbik I., Stasikowska-Kanicka O., Boncela J., Wagrowska-Danilewicz M. and Kowalska M.A. Snail Overexpression Alters the microRNA Content of Extracellular Vesicles Released from HT29 Colorectal Cancer Cells and Activates Pro-Inflammatory State In Vivo. Cancers, 13, 172 (2021); https://doi.org/10.3390/cancers13020172

Immunofluorescence staining and confocal microscopy in the cellular interaction and cell biology studies.

Participation in scientific projects gave me the possibility to get new abilities, to now new methods, and establish cooperation, which broader my scientific interests. In some project I was engaged to plan, perform and analyse confocal imaging. One of the most fruitful collaboration was with prof. Przemysław Lewkowicz (Department of Immunogenetics, Medical University of Lodz). We published together four, high impacted papers. A confocal image of neutrophils producing IL-10, captured by me, was appreciated by the editors from Nature Publishing Group and illustrated the article and the cover of the issue

Articles concerning this part of research

- Kiełbik M., Szulc I., Brzezińska M., Bednarska K., **Przygodzka P.,** Sułowska Z., Nowak M., Klink M. Nitric oxide donors reduce the invasion ability of ovarian cancer cells in vitro. Anticancer Drugs, Nov; 25(10), 1141-1151 (2014); doi: 10.1097/CAD.0000000000000149

- Michalski M., St Swierzko A., Lukasiewicz J., Man-Kupisinska A., Karwaciak I., **Przygodzka P.**, Cedzynski M. Ficolin-3 activity towards the opportunistic pathogen, Hafnia alvei. Immunobiology, Jan; 220(1), 117-123 (2015); doi: 10.1016/j.imbio.2014.08.012

- Lewkowicz N., Mycko M.P., **Przygodzka P.**, Ćwiklińska H., Cichalewska M., Matysiak M., Selmaj K., Lewkowicz P. Induction of human IL-10-producing neutrophils by LPS-stimulated Treg cells and IL-10. Mucosal Immunology, Mar; 9(2), 364-378 (2015); doi: 10.1038/mi.2015.66

- Piątek P., Domowicz M., Lewkowicz N., **Przygodzka P.**, Matysiak M., Dzitko K., Lewkowicz P. C5a-Preactivated Neutrophils Are Critical for Autoimmune-Induced Astrocyte Dysregulation in Neuromyelitis Optica Spectrum Disorder Frontiers in Immunology, July 23 (2018); doi.org/10.3389/fimmu.2018.01694

- Lewkowicz N., Piątek P., Namiecinska M., Domowicz M, Bonikowski R., Szemraj J., **Przygodzka P.**, Stasiołek M. and Lewkowicz P. Naturally Occurring Nervonic Acid Ester Improves Myelin Synthesis by Human Oligodendrocytes. Cells, 8, 786 (2019); doi:10.3390/cells8080786

- Piątek P., Namiecinska M., Domowicz M., **Przygodzka P.**, Wieczorek M., Michlewska S., Lewkowicz N., Tarkowski M. and Lewkowicz P. MS CD49d+CD154+ Lymphocytes Reprogram Oligodendrocytes into Immune Reactive Cells Affecting CNS Regeneration. Cells, 8, 1508 (2019); doi:10.3390/cells8121508

5. Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions

After the conferment of the PhD degree in 2005, I began my post-doctoral fellowship in the Department of Medical Biochemistry and Biophysics, Umeå University, Sweden, in the group of prof. Tor Ny. Under the supervision of dr Malgorzata Wilczynska, I learned new experimental methods and took part in workshops and seminars. I was involved in the research project financed by The Cancer Research Found in Norrland, titled: "Physiology and pathology of intracellular serpins in hematopoietic differentiation and leukemia development." I studied the role of the serpin, bomapin, in cancer progression in the laboratory with an established reputation in the field of serpin research (notably, inhibitors of plasminogen PAI-1, PAI-2). Bomapin is a nuclear protein expressed in bone marrow progenitor cells as well as leukemic cells, not expressed in fully differentiated normal leukocytes. We showed that bomapin was involved in processes essential for normal hematopoiesis and for the prevention of

leukemic transformation. It sensitizes myeloid progenitor cells to growth conditions and regulates their proliferation and/or apoptosis. Results concerning bomapin were presented during the poster session at the *XIth International Workshop on Molecular and Cellular Biology of Plasminogen Activation, Satlsjobaden, Sztokholm, Sweden 2007* and summarised in the research article (***Przygodzka P.***, *Ramstedt B., Engel T., Larsson G., Wilczynska M. Bomapin is a redox-sensitive nuclear serpin that affects responsiveness of myeloid progenitor cells to growth environment. BMC Cell Biology 2010; 11: 30; doi: 10.1186/1471-2121-11-30*). Post-doctoral training in the international team using a wide spectrum of experimental methods allowed me to gain new abilities, get familiar with new experimental approaches and become a more self-directed scientist.

6. Presentation of teaching and organizational achievements as well as achievements in popularization of science or art

a) Dissertation co-advisor

In 2012 I was involved in the Maestro project (NSC, Poland). Results obtained within the project inspired the subsequent studies of neuromedin U in CRC and delivered preliminary data for my own research project proposal. **In 2016, the National Science Centre (NSC, Poland) provided funds for the Sonata Bis 6 project titled: "Neuromedin U, new potential regulator of colorectal cancer metastasis mechanisms".** Within the project, I employed two PhD students. Dr Kamila Soboska defended her PhD and on 18-10-2022 obtained a doctoral degree in medical sciences (Supervisor: dr hab. Joanna Boncela prof. IMB PAS; Co-advisor: dr Patrycja Przygodzka). MSc Ewelina Sochacka applied with success to the BioLAB program (Fulbright Poland) and after completing the experimental part of the project went for a year fellowship at the Department of Cell Biology, the University of Virginia with Prof. Saurabh Kulkarni as a leader. Currently, MSc Ewelina Sochacka is preparing her doctoral thesis (Supervisor: dr. hab. Joanna Boncela prof. IMP PAS; Co-advisor: dr. Patrycja Przygodzka).

dr. Kamila Soboska – investigator in the Sonata Bis 6 project (PI: P. Przygodzka) 2016-2022,
Student of Doctoral Study in Molecular Genetics, Cytogenetics, and Medical Biophysics at the University of Lodz. Dissertation title: „Neuromedin U (NMU) as a regulator of colorectal cancer microenvironment regulator" – Cellular Signaling Lab, Institute of Medical Biology, PAS, Lodz. Doctoral conferment on 18-10-2022. Supervisor: dr hab. Joanna Boncela prof. IMB PAS
Co-advisor: dr Patrycja Przygodzka

M.Sc. Ewelina Sochacka – investigator in the Sonata Bis 6 project (PI: P. Przygodzka) 2016-2022,
Student of Doctoral Study in Molecular Genetics, Cytogenetics, and Medical Biophysics at the University of Lodz. Dissertation title: „The role of neuromedin U (NMU) in the regulation of colorectal cancer cell migration" – Cellular Signaling Lab, Institute of Medical Biology, PAS, Lodz.
Supervisor: dr hab. Joanna Boncela prof. IMB PAS; Co-advisor: dr Patrycja Przygodzka

b) Tutor – student practice

Agata Jackiewicz	Lodz University of Technology, Faculty Biotechnology and Food Science, Biotechnology, full-time studies (M.Sc.), Ist year, II degree Term of practice: 17.09.2012 – 28.09.2012
Agnieszka Godos	Lodz University of Technology, Faculty Biotechnology and Food Science, Biotechnology, full-time studies (M.Sc.), Ist year, II degree Term of practice: 17.09.2012 – 28.09.2012
Maciej Smolarz	Medical University of Lodz, Faculty of biomedical science Biotechnology, full-time studies (M.Sc.), Ist year, II degree Term of practice: 01.07.2013 – 31.07.2013
Edyta Zub	Lodz University of Technology, Faculty Biotechnology and Food Science, Biotechnology, full-time studies (M.Sc.), Ist year, II degree Term of practice: 12.09.2016 – 23.09.2016

c) Organisation of Science Festival

I participated in the organization of "open days" in the Institute during the Science Festival in 2010-2016. I prepared workshops for visitors.

d) Scientific Board member

In the years 2012-2015 and 2016-2019 I represented adjuncts and professor assistants in the Scientific Board of the Institute of Medical Biology of PAS. I performed the function of the Secretary of the Scientific Board during these years.

e) Participation in the scientific conferences

Before the conferment of the PhD degree

1. Jerczynska H., Pawlowska Z., Szemraj J., **Baranska P.**, Swiatkowska M., Cierniewski C.S. Wpływ peptydów natriuretycznych na ekspresję inhibitora aktywatora plazminogenu typu I (PAI-1) w komórkach śródbłonka ludzkiego. Konferencja Acta Angiologica, 8, 53, 2002, Kraków 2002; poster

2. Pawlowska Z., Jerczynska H., Szemraj J., **Baranska P.,** Swiatkowska M., Cierniewski C.S. Natriuretic peptides reduce plasminogen activator inhibitor-1 expression in endothelial cells by the inhibition of its promoter activity. European Life Scientist Organisation (ELSO) Congress, Nicea 2002; poster

3. Jerczynska H., Pawlowska Z., Szemraj J., **Baranska P**., Swiatkowska M., Cierniewski C.S. Inhibition of PAI-1 promoter activity by C-type peptide in human endothelial cells. International Symposium on Promotion of International Cooperation in Eastern and Southern Europe in the Field of Medicinal Biotechnology, 58, Lodz 2002; poster

4. **Baranska P.,** Pawlowska Z., Jerczynska H. Cierniewski C.S. The role of vascular endothelial growth factor in an early phases of proangiogenic changes in human endothelial cells. XIXth Congress of the International Society on Thrombosis and Haemostasis, Birmingham 2003; CD067, J. Thromb. Haemost., suppl. 1 July 2003; poster

5. Pawlowska Z., Jerczynska H., **Baranska P.,** Swiatkowska M., Cierniewski C.S. Downregulation of PAI-1 expression by natriuretic peptides is mediated by MAPK cascade in human endothelial cells. XIXth Congress of the International Society on Thrombosis and Haemostasis, Birmingham 2003, P1275, J. Thromb. Haemost., suppl. 1 July 2003; poster

6. **Baranska P**., Jerczynska H., Koziolkiewicz W., Pawlowska Z., Cierniewski C.S. Induction of the angiogenic phenotype in HUVEC by VEGF. Acta Bioch. Pol., 50, 185, suppl. 1/2003; poster

7. Pawlowska Z., **Baranska P**., Jerczynska H., Koziolkiewicz W., Cierniewski C.S. Cellular machinery for protein synthesis is highly upregulated in VEGF-activated human endothelial cells. IV Interdisciplinary Euroconference on Angiogenesis. p51, Helsinki, Finland 2004; poster

8. Pawlowska Z., **Baranska P.,** Jerczynska H., Koziolkiewicz W., Cierniewski C. S. Upregulation of cellular machinery for protein synthesis upon activation of human endothelial cells by VEGF. 29th Meeting of the Federation of the European Biochemical Societies, Warsaw, 2004, Europ. J. Biochem. The FEBS Journal, 271, suppl. 1, 2004; poster

9. Jerczynska H., **Baranska P.,** Pawlowska Z., Walkowiak B. Growth of endothelial cells at surfaces of selected biomaterials. 2nd Summer School: Biomedical applications of carbon surfaces. 10, 2004, 21st - 25th of September 2004, Lodz / Wysowa; poster

10. Pawlowska Z., Komorowski J., Jerczynska H., Siejka A., **Baranska P.,** Lawnicka H., Stepien H. Thalidomide Affects Early Phases of VEGF Stimulated Angiogenesis in Human Endothelial Cells. a/ The FEBS Journal, 272, suppl. 1, 043P, Budapest 2005 b/ J. Thromb. Haemost., 3, suppl. 1, P0555, Sydney 2005; poster

11. Walkowiak B., Jerczynska H., **Baranska P**., Koziolkiewicz W., Pawlowska Z. Interaction of endothelial cells with selected biomaterials causes changes in protein expression profile. The FEBS Journal, 272, suppl. 1, Budapest 2005; poster

12. Walkowiak B., Jerczynska H., **Baranska P.,** Koziolkiewicz W., Pawlowska Z. Protein expression profile of endothelial cells is changed by contact with selected biomaterials. Konferencja Biologii Komórki, Łódź 2005, Folia Histochemica et Cytobiologica, 43, suppl. 1, S5/2, 2005; poster

After the conferment of the PhD degree:

13. **Przygodzka P**., Olausson B., Tengel T., Larsson G., Wilczynska M. Bomapin Is a Redox-regulated Serpin which Stabilizes Retinoblastoma Protein during Apoptosis and Increases Proliferation of Leukemia Cells. XIth International Workshop on Molecular and Cellular Biology of Plasminogen Activation, Satlsjobaden, Sztokholm, Sweden 2007; poster

14. Olausson B., **Przygodzka P.,** Dahl L., Carlsson L., Wilczynska M. The Serpinb8 Is Alternatively Spliced to the Known Long Form and a Novel Short Form. XIth International Workshop on Molecular and Cellular Biology of Plasminogen Activation, Satlsjobaden, Sztokholm, Sweden 2007; poster

15. **Przygodzka P**., Boncela J., Cierniewski C.S. Nuclear matrix protein matrin 3 as a possible nuclear processes regulator. 21st International Union of Biochemistry and Molecular Biology International Congress and 12th Federation of National Societies of Biochemistry and Molecular Biology in the Asian and Oceanian Region Congress of Biochemistry and Molecular Biology: "Biomolecules for Quality of Life" Zjazd Międzynarodowego Towarzystwa Biochemii i Biologii Molekularnej, Szanghaj, Chiny 2009; poster

16. Boncela J., **Przygodzka P**., Cierniewski C.S. Interaction of PAI-1 and proteasome in endothelial cells. 21st International Union of Biochemistry and Molecular Biology International Congress and 12th Federation of National Societies of Biochemistry and Molecular Biology in the Asian and Oceanian Region Congress of Biochemistry and Molecular Biology: "Biomolecules for Quality of Life" Zjazd Międzynarodowego Towarzystwa Biochemii i Biologii Molekularnej, Szanghaj, Chiny 2009; poster

17. Boncela J., Papiewska-Pajak I., **Przygodzka P.,** Wyroba E., Cierniewski C.S. PAI-1 and PAI-2 inhibit proteasome activity favoring proapoptotic signaling in endothelial cells. XXIII International Congress of the International Society of Thrombosis and Haemostasis, 23-28 lipca 2011, Kyoto, Japonia; poster

18. Mena M.P., Papiewska-Pajak I., Kozaczuk A., Stasiak M, Boncela J., **Przygodzka P**., Cierniewski C.S. Role of the Nuclear Factor of Activated T cells (NFAT) in Inducing a Pro-angiogenic Profile of Integrin Expression in Endothelial Cells. 2nd Congress of Biochemistry and Cell Biology, 46th Meeting of the Polish Biochemical Society and 11st Conference of the Polish Cell Biology Society; 5-9 września 2011, Kraków, Acta Biochimica Polonica (2011), Vol. 58, supl. 2, p. 149; poster

19. **Przygodzka P.,** Boncela J., Wyroba E., Papiewska-Pajak I. and Cierniewski C.S. Secretion of PAI-2 from endothelial cells activated with inflammatory stimuli. 22nd International Union of Biochemistry and Molecular Biology & 37th FEBS Congress Seville, Spain September 4–9, 2012 FEBS Letters Volume 279 Supplement 1 September 2012; poster

20. Mena M.P., Kozaczuk A., Papiewska-Pajak I., **Przygodzka P.,** Stasiak M., Boncela J., Cierniewski C.S. Nuclear Factor of Activated T cells (NFAT) as a Switch Inducing a Pro-angiogenic Integrin Profile in Endothelial Cells. 47th Congress of the Polish Biochemical Society and Polish-German Biochemical Societies Joint Meeting, 2012. 11-14 września 2012, PoznańActa Biochimica Polonica (2012), Vol.59 suppl. 3, p. 131; poster

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https://www.tandfonline.com/doi/abs/10.1080/20013078.2018.1461450 ISEV 2018 abstract book Page 233 PS07.06; poster

34. Sochacka E., Soboska K., **Przygodzka P.** Charakterystyka linii komórkowych raka jelita grubego i odbytnicy pod względem ekspresji neuromedyny U. IV Ogólnopolska Konferencja Doktorantów Nauk o Życiu BIOOPEN ŁÓDŹ, 24 – 25.05.2018 r. Poster P.98; poster

35. Soboska K., Sochacka E., **Przygodzka P.**, Boncela J. Neuromedin U as a potential colorectal cancer microenvironment modulator. European Association for Cancer Research (EACR) 2nd joint EACR-MRS Conference "Seed and Soil - mechanisms of metastasis" 07-09 October 2019 Berlin, Germany; poster session, poster no. 104, Abstract book page 139; poster

36. Sochacka E., Soboska K., **Przygodzka P.**, Boncela J. Neuromedin U is implicated in colorectal cancer cells invasiveness. European Association for Cancer Research (EACR) 2nd joint EACR-MRS Conference "Seed and Soil - mechanisms of metastasis" 07-09 October 2019 Berlin, Germany; poster session, poster no. 105, Abstract book page 140; poster

37. Soboska K., Sochacka E., Pacholczyk M., Papiewska-Pająk I., Braun M., Kiełbik M., Boncela J., **Przygodzka P.** Neuromedin U as a modulator of CRC microenvironment – induction of colorectal cancer cells and macrophages motility. 18th Biennial Congress of the Metastasis Research Society, November 15 – 17, 2021; poster no. 75; poster

38. Soboska K., Sochacka E., Pacholczyk M., Papiewska-Pająk I., Braun M., Kassassir H., Kiełbik M., Boncela J., **Przygodzka P.** Modulation of colorectal cancer microenvironment by neuromedin U secreted by cancer cells - the importance of functional tumour associated cells in cancer invasiveness research. EACR conference Seed and Soil: In Vivo Models of Metastasis Virtual Event, Worldwide: 25 - 26 January 2022 POSTER NO. 21; poster

7. Apart from information set out in 1-6 above, the applicant may include other information about his/her professional career, which he/she deems important.

a) Prizes

- Discretionary award for exemplary work as a Secretary of the Scientific Board of the Institute awarded by the Director of Institute of Medical Biology PAS - 2012
- Discretionary award for scientific achievements awarded by the Director of Institute of Medical Biology PAS 1° - 2016, 2017, 2018, 2020, and 2° - 2019
- Discretionary award for the article from the JCR list; awarded by the Director of Institute of Medical Biology PAS – 2017
- Discretionary award for the publication cycle entitled: "Inflammation as a common factor of diseases of civilization (type 2 diabetes, depression) – epigenetics and therapy" awarded by the President of Medical University of Lodz - 2021

b) Honours

I performed confocal imaging of neutrophils producing IL10 as a part of cooperation with prof. P. Lewkowicz (Medical University of Lodz), and the picture found appreciation of the Nature Publishing Group Editors and was located on the cover of Mucosal Immunology vol. 9(2) in March 2016.

c) National collaboration

Confocal imaging was the main field of collaboration with prof. Przemysław Lewkowicz (Department of Immunogenetics, Medical University of Lodz) as it was mentioned above. We published together four, high impacted papers. Besides fruitful cooperation inside our project team, we were able to expand our NMU studies thanks to the collaboration with other researchers, i.e. dr Marcin Pacholczyk, an expert in data analysis (Department of Systems Biology and Engineering, Silesian University of Technology, Gliwice) and dr Marcin Braun, an expert in immunohistochemistry (Department of Pathology, Chair of Oncology, Medical University of Lodz).

d) International collaboration

During my post-doctoral fellowship in the Department of Medical Biochemistry and Biophysics, Umeå University, Sweden, I had an opportunity to collaborate with Prof. Tor Ny and his group in the field of serpins biology, with Prof. Richard Lundmark in the field of cell immunofluorescence staining and confocal microscopy, and with the group of Prof. Andrei Chabes in the field of flow cytometry. Back in Poland I prepared and submitted a grant proposal in *Pomost* call (Foundation for Polish Science) titled: "Role of matrin 3 in neuronal function and Alzheimer's disease" with planned collaboration with Prof. Ludmilla Morozova-Roche from Umeå University. Unfortunately, the project was not accepted for funding. I still maintain contact with a group of researchers from Umeå University and they supported us in our projects e.g. Prof. M. Wilczyńska provided us with specific plasmids for controlled serpins overexpression.

Neuromedin U studies within Sonata Bis 6 project generated new unexpected problems and in order to solve them, I looked for the help of an expert in active peptides chemistry. I wrote to Prof. Steven Ballet (Vrije Universiteit Brussel), and he appeared to be enthusiastic about our observations concerning NMU and offered to provide us NMURs agonists, which enabled the studies of endogenously expressed receptors activity in CRC cells. Our collaboration resulted in high impacted publication and new project proposal preparation (Opus21, in progress).

e) Courses and workshops

12.2003	Flow cytometry methods - research and diagnostic – University of Lodz
11.2007	Molecular cloning - Umeå University, 901 87 Umeå, Sweden
04.2010	Gene expression School - Applied Biosystems, Warszawa
10.2011	Self-presentation and public speaking – FNP, Warszawa
10.2014	Project Management in a Nutshell - Ster dla B+R, Warszawa
05.2020	Prism Academy – GraphPad – on-line
06.2020	EndNote Online Course - Become an Expert Today – on-line

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