Autoreferat

1. Name and surname

   Magdalena Narajczyk

2. Diplomas, degrees – with the name, place and year of acquisition and the title of doctoral dissertation

   Master of Science – Faculty of Biology, Geography, Oceanology, University of Gdańsk, June 2002

   The degree of doctor of biological sciences in the field of biology - Faculty of Biology, Geography, Oceanology, University of Gdańsk, July 2007. Title of PhD thesis: The mechanism of unidirectional and bidirectional initiation of DNA replication at the *ori*λ region".

3. Academic carrieer

2007-2009	assistant, University of Gdańsk, Faculty of Biology, Department of Molecular Biology
2009-2010	lecturer, University of Gdańsk, Faculty of Biology, Department of Molecular Biology
2010 - now	lecturer, University of Gdańsk, Faculty of Biology, Laboratory of Electron Microscopy
03.2011 – now	headmaster of Laboratory of Electron Microscopy, Faculty of Biology, University of Gdańsk

4. Indication of achievement under art. 16 paragraph 2 of the act of 14 March 2003 on academic degrees and titles and art degrees and titles:

   a) title of scientific achievement
   Electron microscopy methods used in biomarker analysis of mucopolysaccharidoses – a group of lysosomal storage disorders

   b) (autor/autors, title/titles of publications, year, name of publishers)

   [1] Malinova V., Węgrzyn G., **Narajczyk M.** (2011) The use of elevated doses of genistein-rich soy extract in the gene expression-targeted isoflavone therapy for Sanfilippo disease patients. *Journal of Inherited Metabolic Disease Reports*, 5: 21-25 (IF 2011 = 3,577, MS&HE = 25)

[2] de Ruijter J., Valstar M.J., **Narajczyk M**., Węgrzyn G., Kulik W., Ijlst L., Wagemans T., van der Wal W.M., Wijburg F.A. (2012) Genistein in Sanfilippo disease: a randomized controlled cross-over trial. *Annals of Neurology*, 71:110-120 (IF 2011 = 11,089, MS&HE = 50)

[3] **Narajczyk M.,** Tylki-Szymańska A., Węgrzyn G. (2012) Changes in hair morphology as a biomarker in gene expression-targeted isoflavone therapy for Sanfilippo disease. *Gene,* 504: 292-295 (IF 2011 = 2,341, MS&HE = 25)

[4] Kloska A., **Narajczyk M.,** Jakóbkiewicz-Banecka J., Grynkiewicz G., Szeja W., Gabig-Cimińska M., Węgrzyn G. (2012) Synthetic genistein derivatives as modulators of glycosaminoglycan storage. *Journal of Translational Medicine,* 10: 153 (IF 2011 = 3,47, MS&HE = 35)

[5] **Narajczyk M.,** Moskot M., Konieczna A. (2012) Quantitative estimation of lysosomal storage in mucopolysaccharidoses by electron microscopy studies. *Acta Biochimica Polonica,* 59: 693-696 (IF 2011 = 1,491, MS&HE = 15)

c) Discussion of research aims of above mentioned publications and achieved results with discussion on their possible use

Scientific objective of these publications was to estimate the effectiveness of electron microscopy methods in biomarker analysis of storage diseases, especially in mucopolysaccharidoses. Lysosomal storage disorders are the group of genetically - determined metabolic diseases. Mutation in one of genes coding acid hydrolase leads to a genetic defect, which revealed in inhibition of decomposition of organic compounds. Results of their accumulation in cells lead to dysfunction of cells. Despite wide variety of these disorders, there are features in common. There are multi-organ pathological change, progressive and serve disease, and also premature death.

Mucopolysaccharidoses (MPS) are rare lysosomal storage disorders. Inherited defects of glycosaminoglycans (GAG) metabolism cause accumulation of undegraded specific organic compounds. Glycosaminoglycans are compounds, which occur in many tissues of mammalian organism. They are present in extracellular space and combined with cell membrane, they are involved in development of the placenta, participate in cell signaling pathways, limit the ability of fibroblast grow factor to bind to receptors. Most of GAGs occur as peptidoglycan, covalently bind with proper protein. Degradation of

glycosaminoglycans proceeds in lysosomes, which is mediated by specific enzymes. Deficiency or lack in an activity of one of enzymes leads to accumulation of GAGs in lysosomes as well as outside of cells. This process leads to disorder in the structure and functioning of cells and consequently to characteristic clinical symptoms.

There are eleven types and subtypes of mucopolysaccharidoses, according to deficiency of enzyme in patients' cells. Due to impairment in function of most of organs and progressive character of this disease, the average life span is only several years. Moreover, in most cases diagnosis is made in age of few years, because mucopolysaccharidoses reveal a bit later than most genetic diseases.

Since the development of science and technology, enzyme replacement therapy can be used in treatment of MPS type I, type II and VI. This treatment is based on intravenous administration of human recombinant enzyme to the patient, which is then transported into cells and localized in lysosomes. Such an addition of enzyme, results in degradation of accumulated glycosaminoglycans, and in result clinical improvement of patients. Unfortunately, due to the blood-brain barrier this therapy in ineffective in mucopolysaccharidoses with affected central nervous system, for example for MPS type I (Hurler disease) and type III (Sanfilippo disease). Substrate reduction therapy is one of the alternative methods for MPS treatment. A compound which could be used in such therapy is genistein (4',5,7-trihydroxy-3-fenylochromen-4-on) – compound from a group of isoflavones. Genistein can indirectly regulate the expression of genes acting as an inhibitor of activity of EGF receptor kinase. It was shown previously that genistein added to fibroblast cell culture decreases efficiency of GAG production. Additionally, this isoflavone indirectly inhibits GAG synthesis by blocking EGFR phosphorylation, which causes a decrease in gene expression of GAG biosynthesis enzymes. Adding genistein to MPS fibroblast cell culture resulted in disappearance of abnormal intracellular structures. Moreover, this isoflavone can cross the blood-brain barrier, and what is important, genistein is safe to use. Due to high costs of enzyme replacement therapy and limited availability of treatment for all types of mucopolysaccharidoses, it is very important to find other treatments for MPS. Due to variety of symptoms and different progress of illness in MPS patients, precise determine the effectiveness of therapeutic method is very difficult. On the other hand, estimation the effectiveness of treatment due to analysis of proper biomarkers is very important, especially considering small group of patients involved in the study.

Due to reports of positive effect of genistein on MPS patients, I participated in the clinical trial with scientists from the Netherlands. In this trial, we studied the influence of genistein on Sanfilippo patients

(publication 2). Randomize, crossover, double blinded, placebo-controlled clinical trial with 30 patients - divided on two groups, each consisting of 15, was performed. Patients were treated with first genistein (10 mg/kg/day) and then placebo, and vice versa. During the study, I analysed hair morphology by scanning electron microscopy in three periods of time: at the beginning of the trial, after 6 months and 13 months of trial (after 6 months of trial it was 1 month of washout, with neither genistein nor placebo given to patients). During the microscopic analysis, probes were blinded, which allowed me to maintain objectivity. Despite the significant amount of hair, statistical analysis did not show any significant differences between control and genistein groups of patients. It is possible that time of genistein administration was too short or the dose of 10 mg/kg/day was too low to observe significant therapeutical effects.

During the clinical trial in the Netherlands I have started cooperation with Dr Vera Malinova from Charles University in Praha (Czech Republic). In this cooperation, I analysed, with the use of scanning electron microscopy, hair morphology from MPS type III patients (Sanfilippo disease) (publication 1). At the beginning of our cooperation, Dr Malinova's patients had taken genistein at concentration of 5 mg/kg/day, however, considering information obtained during the trial with patients from the Netherlands, we have increased the dose of genistein to 15 mg/kg/day. Hair samples were taken in total periods of 16-22 months (in different patients) in intervals between 3 to 6 months. I have observed a significant improvement in hair morphology analysed in electron microscopy after increasing genistein concentration. Nevertheless, it should be noted that only 6 patients were examined, and studies with larger number of patients are desirable.

Considering suggestions mentioned above, I performed microscopy analysis of hair taken from 35 patients, which were treated with genistein at the dose of 5 and 15 mg/kg/day for 12 months (publication 3). Through studies using scanning electron microscopy, I indicated that improvement of hair morphology was noted in 26 out of 35 patients. I did not observe any changes in hair morphology in 8 patients and I noticed worsening only in 1 patient (I spotted slight abnormal changes in the hair structure). I observed improvement in hair morphology in all patients for which the dose of genistein was higher. These studies demonstrated the effectiveness of microscopy analysis of hair morphology as a biomarker during treatment of MPS type III patients. It is very important, because this assay has a non-invasive character.

I was interested in possibility of combining two mucopolysaccharidose treatment, namely enzyme replacement therapy and substrate reduction therapy. Combination of these two therapies allows reducing the cost of treatment, and also due to properties of

genistein, neurodegeneration decrease. Due to the early stage of genistein, I decided to perform microscopy analysis with fibroblast cell culture from MPS type I patient (publication 5). For my research I used MPS type I cell culture because of the availability enzyme replacement therapy in this disease – enzyme α-L-iduronidase. I reduced enzyme concentration to 50% (relative to current dose in the therapy) and added genistein at the dose of 30 μM. As a control, I used human dermal fibroblast adult line (HDFa) and MPS type I cells cultivated only in the medium (DMEM). Through transmission electron microscopy techniques, in cells with combination of treatment (genistein and enzyme), I observed almost four times reduction in the level of glycosaminoglycans relative to MPS type I cells control, and two times decrease in the level of GAG relative to MPS type I cells treated with the enzyme at concentration corresponding to current dose in the therapy. These experiments showed that the use of both therapies at the same time should be next step in MPS treatment. Moreover electron microscopy experiments prove the validity of these methods with biochemical research in estimation of changes in patient cells.

Due to therapeutic possibilities of genistein, I was curious of properties of synthetic derivatives of this isoflavone (publication 4). I analysed, by using transmission microscope, cells from MPS type III patients, subtype A and B, treated with seven different genisteinderivatives. In ultrastructure of cells, I noticed significant decrease of glycosaminoglycans' level relative to control. Microscopic examination showed changes in cells morphology, decrease of single lamellar lysosomal structures, as well as other complex lysosmal structures. Statistical analysis showed significant differences in most of the derivatives. These experiments demonstrated the possibility to use other synthetic derivatives of genistein in MPS treatment.

In summary, my scientific achievement, showed in this habilitation, consist of following discoveries:

- hair morphology analysis with scanning electron microscopy could be used as a non-invasive method for monitoring of treatment for MPS;
- cell ultrastructure analysis with transmission electron microscopy might be used as a biomarker equally with biochemical methods in mucopolysaccharidoses;
- GAG level decrease in cells treated with enzyme and genistein suggests the possibility to combine both of treatments in MPS;
- GAG level decrease in cells treated with synthetic derivatives of genistein might have further use in MPS therapy.

5. Discussion of other scientific and research achievements

I started my Master's degree studies in Institution of Biology of Faculty of Biology, Geography and Oceanology at University of Gdańsk in 1997. I performed research for my thesis at Department of Molecular Biology, and tutors of my work were Dr Sylwia Barańska and Prof. Grzegorz Węgrzyn. Then, I became interested in DNA replication, especially in directionality of this process. For my analyses, I used natural plasmid pIGRK-1, which was isolated from clinical strain of *Klebsiella pneumoniae*. Experiments allowed determination of directionality of DNA replication, which I used, was DNA two dimensional agarose gel electrophoresis and electron microscopy techniques. According to these experiments, I showed second *origin* site for DNA replication of pIGRK-1 plasmid and I determined directionality of replication forks. These results were the basis of my thesis, which I defended in June 2002.

After graduation, between 2002-2007, I became a PhD student at Environmental Doctoral Studies in Biology and Oceanology at University of Gdańsk. I still continued researches in DNA replication and its directionality. Generally known model assumes that initiation of the σ mode of bacteriophage λ DNA replication is preceded by one round of unidirectional θ replication initiated at *oriλ*. That is why my interests focused on factors, which potentially could be involved in regulations of DNA replication during the lytic cycle. The results of this research were included in publications from point IIA-1, 2 (appendix 3).

According to 2D agarose gel electrophoresis of λ DNA and density shift experiments, I showed that ClpPX protease does not influence the replication mode of λ DNA, as was proposed by Prof. Żylicz. Using electron microscopy techniques I confirmed my results (publication IIA-1).

I also showed, that SeqA protein influences the replication mode of λ DNA (publication IIA-2). I proved that SeqA protein is involved in the stability of heritable λ replication complex. Experiments were performed using Δ*relA* strains and in amino acid-starved bacteria. I estimated the stability of the λO protein and showed that the SeqA protein influences stability and function of the heritable complex.

Above results were included in my dissertation "Mechanism of unidirectional and bidirectional initiation of DNA replication at *oriλ*", which I performed under supervision of Prof. Grzegorz Węgrzyn. I received my Ph.D. degree in July 2007. Due to my interests and knowledge in electron microscopy techniques, I was hired in Laboratory of Electron Microscopy, Department of Molecular Biology at University of Gdańsk in May 2007.

After defense of my dissertation my scientific interests focused on lysosomal storage disorders, especially on mucopolysaccharidoses (MPS). Despite publications mentioned in the first part of my autoreferat, I analyzed the ultrastructure of fibroblast cells isolated from patients with MPS type III treated with flavonoids (genistein, daidzein, kaempferol and other) (publication IIA- 3, 7). Performing electron microscopy research, I showed and confirmed results obtained from the biochemical experiments. I noticed significant level of decrease of glycosaminoglycans (GAG) in cells treated with genistein relative to control experiments. Moreover, microscopy analysis confirmed that genistein inhibits GAG synthesis through inhibition of EGFR phosphorylation (publication IIA-3). These data were the basis of my further research with genistein as a potential treatment for MPS.

In subsequent studies, I analyzed by microscopy cells treated with other flavonoids: naringenin, daidzein, kaempferol, apigenin (publication IIA-7). In the ultrastructure of examined cells, I noticed significant morphological changes, especially in cells treated with daidzein and kaempferol. This study may be continued in further experiments, and examined flavonoids could be another therapeutic possibility in substrate reduction therapy.

I performed analogical experiments during research, which were published in work from point IIA-10. I analyzed MPS type I and III cells, which were at the same time incubated with two specific siRNAs to inhibit gene expression of glycosaminoglycans. This procedure has been proposed as a potential treatment for MPS. Even if microscopic experiments showed some decrease in levels of glycosaminoglycans in cells, changes were not statistically significant.

I was also involved in co-writing publications about potential possibilities for MPS treatment focused on substrate reduction therapy with genistein (publication IIA-5, 6, 8). Additionally I am still involved in phage morphology research, where phages were isolated from different environments. The analyses of differences in phages were shown in publications IIA-4, 9.

In view of my knowledge about different electron microscopy techniques, both transmission and scanning microscopy, two years ago I became a chairman of Laboratory of Electron Microscopy, a an independent unit at Faculty of Biology, University of Gdańsk. In the nearest future I am going to examine with transmission electron microscopy the influence of combined enzyme replacement therapy and substrate reduction therapy on glycosaminoglycans accumulation in cells from MPS type VI patients. I am also going to continue the analyses of hair morphology from patients treated with genistein.

Magdalena Narajczyk
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