Univerza v Mariboru

FRAMEWORK PROGRAMME OF EARLY STAGE RESEARCHER TRAINING[1]

1. BASIC DATA

Mentor's name and surname	**Assoc. Prof. dr. Tomaž Langerholc**	Mentor's register number at ARIS (SICRIS)**:**	**23574**
Mentor's e-mail:	tomaz.langerholc@um.si	Mentor's tel. no.:	+386 (0)2 3209054
Research programme (RP) leader's name and surname:	Prof. dr. Dejan Škorjanc	RP leader's register number at ARIS (SICRIS):	01364
Title of research programme:	Research for improvement of safe food and health	RP's Register number at ARIS (SICRIS):	P1-0164
Research organisation (RO) of University of Maribor, where training shall be conducted:	Faculty of Agriculture and Life Sciences	RO Register number at ARIS (SICRIS):	0482
Research field according to ARIS classification:	1.05 Biochemistry and Molecular Biology	Research field according to Ortelius classification (EURAXESS)	7.3. Biochemistry; ID=49

2. DEFINITION OF RESEARCH PROBLEM AND GOALS OF DOCTORAL RESEARCH[2]

Starting point of research task of the early stage researcher and its position in the research programme, where the mentor is included, work hypothesis, research goals and foreseen result with emphasis on an original contribution to science:

Title: Intracellular mechanisms of action of selected antioxidants

Background

Antioxidants have been an important topic in science over the last decade and at the same time an interesting area for the industry and the development of dietary supplements. Various types of synthetic and natural antioxidants are among the most commonly used dietary supplements for humans. The reason for adding antioxidants to the diet is to reduce free radicals, which are

[1] *Term early stage researcher (ESR) is written in male form and used as neutral for women and men.*

[2] *Research and study programme of training have to harmonise with contents of the research programme, where the mentor is a member.*

responsible for damaging biologically important molecules, including cellular DNA, due to their reactivity. Long-term DNA damage can lead to cancer. Recent research has shown that antioxidants also influence cell signalling by altering the redox potential in the cell. It has also been shown that a low redox potential is not always welcome, as it impairs metabolism (energy production in the mitochondria, ATP levels) and reduces the speed of muscle regeneration. Oxidative stress in the cells is a normal phenomenon, but it must be kept within reasonable limits. As antioxidants are weak oxidants, they can also cause oxidative stress, which is why their dosage is important.

Position in the research programme

Antioxidants are important components of our diet because they regulate oxidative stress in the cells and thus influence the ageing process and cell damage. The topic is directly related to the research programme "P1-0164 Raziskave za zagotavljanje varne hrane in zdravja, i.e. Research for improvement of safe food and health" and investigates the mechanisms that influence the optimal dosage of antioxidants in terms of achieving cellular health and thus the whole organism.

Research problem

The antioxidant potential of substances is usually determined using chemical tests (FRAP, ORAC, DPPH, etc.). These simple tests do not reflect the actual effectiveness of antioxidants on the oxidative state in the cell. First of all, antioxidants must be absorbed from food, transported to the tissues and taken up from the blood by active/passive transfer across the cell membrane. Within the cell, they react with certain types of radicals and oxidants (NO, NOO·, O2· -, OH·). At the same time, they are subject to intracellular pharmacokinetics, i.e. excretion from the cell and metabolism.

Working hypotheses

1. Selected antioxidants are absorbed and taken up by the cells to varying degrees
2. The selected antioxidants differ according to the mechanism of intracellular action and signalling pathways
3. The selected antioxidants differ in terms of their effectiveness in regulating the activity of free radicals and thus the consequences of their action.

Research objectives and expected results emphasising the original contribution to science

Knowledge about the intracellular processes and biological activity of antioxidants is scarce, as it is mainly based on chemical methods for measuring antioxidant potential, which do not reflect the real situation in the cells. The main aim of the research is to determine the uptake and intracellular availability, the signalling pathways and the antioxidant mechanisms of action. The new findings would be a further step in the production of optimal mixtures of antioxidants for nutritional purposes, which could be used to produce new functional foods and thus influence public health.

Methodology

The laboratory work will focus on the mechanisms of action and activity of selected antioxidants at the cellular level. Antioxidants from different chemical groups will be selected (vitamin C, α-tocopherol, gallic acid, rasveratrol, selected polyphenols). The latter will be tested for their uptake in selected intestinal epithelial cells as pure substances or by simulating in vitro digestion with artificial digestive juices. The cancer epithelial cell line Caco-2 is used for the experiments. The latter are cultivated in 2D, on a microporous membrane or in the form of spheroids, which are most similar to the physiological state *in vivo*. After the addition of antioxidants to the medium, their time-dependent intracellular concentration is measured. In addition, after removing the antioxidants from the medium, the time-dependent decrease in the intracellular concentration of the antioxidants is monitored. In addition to the intracellular concentration itself, fluorescent dyes (e.g. DCFH-DA) are also used to measure the time-dependent concentration of individual free radicals. The changes in fluorescence over time are quantified using a microplate reader, flow cytometry (which also allows the measurement of cell distribution) and, in the case of spheroids, confocal microscopy. By using different dyes and markers for mitochondria, ATP, redox potential, chromosomal damage (marker H2A) and signalling pathways (NFkb, Jnk, STAT) in the cell and by using flow cytometry and the Agilent Seahorse analyser, we will gain insight into the extensive intracellular events in the presence of antioxidants. Next generation sequencing will allow us to measure changes in gene transcription. The time course of the antioxidant activity of selected antioxidants will be modelled mathematically.

Implementation
Work with cell lines is possible in the FKBV laboratories. Specialised analytical equipment is available at other UM faculties (FKKT, MF), the National Chemical Institute (KI) and the Jožef Stefan Institute (IJS) in Ljubljana. Dr Livija Tušar from IJS can participate in mathematical modelling with neural networks. Laboratory work in foreign laboratories is planned, as we have already collaborated with the Faculty of Medicine in Niš (Serbia) in the field of redox signalling.

3. STUDY PROGRAMME

Foreseen study programme, to which early stage researcher shall be enrolled in academic year 2024/2025:

Agriculture (''Kmetijstvo''), PhD study at the Faculty of Agriculture and Life Sciences

4. DESCRIPTION OF WORK AND TASKS

Studying scientific literature on the topic of the doctoral thesis; planning experiments and their implementation in the laboratory; writing articles; participation in teaching and research activities at the department; writing projects

5. REQUESTED LEVEL OF EDUCATION

Completed master's study (II. Bologna Level)

6. REQUESTED FIELD OF EDUCATION

Natural sciences

7. KLASIUS SRV

18202 Education leading to doctorate of science (third Bologna cycle)/Doctorate of science (third Bologna cycle)

8. KLASIUS P

0512 Biochemistry

9. REQUESTED KNOWLEDGE

Basic knowledge of biochemistry / cell biology is required

10. REQUESTED SPECIAL REQUIREMENTS

No special requirements

11. REQUESTED LANGUAGES

English (writing, speaking) at least B2 level

12. REQUESTED WORK EXPERIENCE

Experience in laboratory work is desirable, especially sterile work techniques, cell cultures

13. FORESEEN POSTDOCTORAL TRAINING

The work is planned until the completion of the PhD

Mentor's signature:

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Digitally signed by Tomaž Langerholc
DN: C=SI, O=Republika Slovenija, OID.2.5.4.97=VATSI-17659957, CN=SIGEN-CA G2
Reason: I am the author of this document
Date: četrtek, 29. februar 2024 09:01:09

Research programme leader's signature:

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Dejan Škorjanc Digitally signed by Dejan Škorjanc
Date: 2024.02.29 10:07:43 +01'00'

Name and surname of Dean or authorised person[3]:
On the authority of the dean, the vice dean prof. dr. Branko Kramberger

Signature of dean or authorised person:

BRANKO KRAMBERGER Digitally signed by BRANKO KRAMBERGER
Date: 2024.02.29 11:30:17 +01'00'

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Place and date:

Hoče 29. 02. 2024

Stamp:

[3] The training program is signed by the dean of the member where the ESR's employment and training will take place.