Summary

The inspiration to undertake the study described in this dissertation were the literature reports suggesting the existence of a strong relationship between the presence of *Fusobacterium nucleatum* (*Fn*) bacteria in colon cancer tissues and colorectal cancer. A significant accumulation of *Fn* population, high level of copper(II) ions and enhanced generation of reactive oxygen species (ROS) in colon cancer tissues has been confirmed until now. ***Thus, the above-mentioned components became the determinant for creation of this dissertation, which is a specific attempt to describe the initial mechanism of free radical processes of colorectal carcinogenesis involving Fn and Cu(II) ions.***

In the first part of the study the characteristics of the Cu(II) ions binding process to seven peptide ligands (six linear and one cyclic), being the FomA protein fragments of *Fn* was presented. The designed ligands contain different amounts of histidyl (His) residues in their sequences (from one to three His residues). Potentiometric titration enabled the determination of the stability constants (logβ) and deprotonation constants (pK_a) of individual Cu(II) species. Moreover, the stoichiometry of the complexes and their stability (logK^* value) which was compared with the Cu(II) complexes found in the literature were determined. In addition, stoichiometry of the complexes was confirmed by mass spectrometry (MS). Complementary spectroscopic methods: electron absorption spectroscopy in the UV-Vis range, circular dichroism spectroscopy (CD) and electron paramagnetic resonance spectroscopy (EPR) allowed to propose coordination modes for individual species. ***The formation of mono-, di- or trinuclear Cu(II) complexes in aqueous solution has been proven depending on the studied system.***

However, the main purpose of the study was to investigate the prooxidative activity of Cu(II) ions after coordination to the peptide ligands in the presence of H_2O_2, ascorbic acid (Asc) or their mixture (H_2O_2 /Asc). After the determination of the coordination modes of Cu(II)-peptide systems at the colon pH (pH range 5.5-7.5), their reactivity in aqueous solution was examined. It was proved that the Cu(II) peptide complexes generate the highest level of ROS (use of electron absorption and emission spectroscopy, EPR spin trapping technique and agarose gel electrophoresis). ***The produced ROS have been identified as hydroxyl radical ($^{\bullet}OH$), singlet oxygen ($^1O_2$) and peroxide radical ($RO_2^{\bullet}$). It has been proven, that Cu(II) complexes do not generate superoxide anion radical ($O_2^{\bullet-}$).*** Furthermore, the generated ROS contribute to single- and double-strand DNA breaks. ***What is more, it has been noticed that the number of imidazole nitrogen atoms coordinated to the Cu(II) ion as well as the cyclization of the peptide chain affect the amount of formed ROS.***

An important aspect of this dissertation is its interdisciplinarity, which goes far beyond the field of chemistry. Therefore, as part of scientific internships, numerous biological (*in vitro* on CT26 colorectal carcinoma cell line) and microbiological (in the presence of the *Fusobacterium nucleatum* subsp. *polymorphum* DSM 20482 bacterial strain) experiments were performed independently.

It has been proven that FomA protein fragments with Cu(II) ions can be a representative model for studying the Cu(II) - bacterial protein - host cell interactions because they do not penetrate the cell (use an inductively coupled plasma mass spectrometry, ICP-MS). Cu(II) peptide complexes stimulate the CT26 cell to intra- and extracellular production of ROS

(using electron emission spectroscopy in the UV-Vis range and EPR spin trapping technique). ***Finally, it has been confirmed that the studied Cu(II) complexes cause intracellular ROS bursts that damage polyunsaturated fatty acids, leading to the formation of malondialdehyde (MDA) inside the cell.***

Moreover, the studies were carried out, which allow to assess the survivability of the anaerobic *Fusobacterium nucleatum* subsp. *polymorphum* DSM 20482 bacteria in the presence of Cu(II) ions and H_2O_2 under oxygen exposure. Bacterial cell death has been proven to occur after 90 minutes. In the final step, it was confirmed that a living organism which is *Fn* in the presence of Cu(II) ions and H_2O_2 is capable to generate ROS (use of the electron absorption spectroscopy in the UV-Vis range).

The above achievements were described in detail in the following publications:

1. **Lesiów M. K., Komarnicka U. K., Stokowa-Sołtys K., Rolka K., Łęgowska A., Ptaszyńska N., Wieczorek R., Kyzioł A., Jeżowska-Bojczuk M.**, „Relationship between copper(II) complexes with FomA adhesin fragments of *F. nucleatum* and colorectal cancer. Coordination pattern and ability to promote ROS production.” **Dalton Transactions**, 2018, **47**, 5445-5458.

2. **Lesiów M. K. (corresponding author), Pietrzyk P., Bieńko A., Kowalik-Jankowska T., “**Stability of Cu(II) complexes with FomA protein fragments containing two His residues in the peptide chain.” **Metallomics**, 2019, **11**, 1518-1531.

3. **Lesiów M. K. (corresponding author), Komarnicka U. K., Kyzioł A., Bieńko A., Pietrzyk P.**, “ROS-mediated lipid peroxidation as a result of Cu(II) interaction with FomA protein fragments of *F. nucleatum*: relevance to colorectal carcinogenesis.” **Metallomics**, 2019, **11**, 2066-2077.

4. **Lesiów Monika K. (corresponding author), Pietrzyk P., Kyzioł A., Komarnicka U. K.** “Cu(II) complexes with FomA protein fragments of *Fusobacterium nucleatum* increase oxidative stress and malondialdehyde level.” **Chemical Research in Toxicology**, 2019, **32**, 2227-2237.

5. **Kędziora A., Lesiów M. K., Krupa K., Korzeniowska-Kowal A., Adamski R., Komarnicka U. K., Stokowa-Sołtys K., Bugla-Płoskońska G., Jeżowska-Bojczuk M.** “Protocol of proceedings with *Fusobacterium nucleatum* and optimization of ABTS method for detection of reactive oxygen species.” **Future Microbiology**, 2020, **15**, 259-271.